# Wall Structure and Wall Loosening. A Look Backwards and Forwards[1]

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The plant cell wall is a strong fibrillar network that gives each cell its stable shape. To enlarge, cells selectively loosen this network, enabling it to yield to the expansive forces generated by cell turgor pressure. Twenty-five years ago, cell wall loosening was mostly explored within the context of rapid auxin-induced growth, particularly in terms of the acid-growth hypothesis (19) proposed independently by Hager in Germany and by Cleland and Rayle in the USA. Discussion of cell wall structure centered on the influential "Albersheim model" first presented by Keegstra et al. (10) (Fig. 1A), and extension growth was widely conceived of as the result of enzymatic hydrolysis of matrix polysaccharides (12).

Today, some new characters such as expansin, xyloglucan endotransglycosylase, and membrane-bound endoglucanases have made an entrance into this scene, forcing a re-evaluation of how wall enlargement is controlled. This brief history summarizes key concepts of cell wall loosening, a topic that inevitably is linked to our view of cell wall structure.

## EVOLVING MODELS OF CELL WALL STRUCTURE

Based on selective enzymatic degradation of sycamore suspension cell walls, Keegstra et al. (10) proposed that matrix polymers, consisting of xyloglucan, pectic polysaccharides, and structural proteins, were covalently linked to form a giant macromolecular network, illustrated in Figure 1A. In this model cellulose is bonded to the matrix via H-bonding to xyloglucans.

This scheme presented several possible sites for wall loosening. For instance, scission of any of the matrix linkages could plausibly to lead to wall extension, since they are arranged in a chain-link series. Keegstra et al. also proposed that low pH might directly weaken the H-bonding between xyloglucan and cellulose, thereby allowing microfibril slippage, but subsequent work from the same laboratory later made this idea untenable.

When later work could not confirm the pectin-xyloglucan linkage, an alternative model gained favor (Fig. 1B). As proposed by Hayashi (9) and Fry (6), cellulose microfibrils may be tethered together directly via long xyloglucan chains. Pectic polysaccharides and structural proteins are imagined as co-extensive, but independent, networks that physically entangle the cellulose-xyloglucan network, but are not covalently bonded to it. Freeze-etch replicas of extracted walls by McCann and colleagues (13) lent support to this model. In addition, work by Whitney and colleagues (24) showed that when cellulosic pellicles produced by *Acetobacter xylinum* were grown in the presence of xyloglucan or similar matrix polysaccharides, the cellulose microfibrils became organized in a way that resembled plant cell walls to a remarkable extent. These and other results implicated hemicelluloses as important physical organizers of wall architecture.

Although the "tethered network" model of Figure 1B is currently the most popular one (2), other variations have been proposed. Talbott and Ray (21) proposed a "multicoat" model in which each microfibril is coated by a series of progressively less-tightly bound polysaccharide layers (Fig. 1C) and the linkage between microfibrils is made indirectly via the lateral (non-covalent) associations between the distinctive polysaccharide layers. Jarvis and colleagues (8) conceived of a more stratified wall in which pectic layers serve as spacers between cellulose-hemicellulose lamellae (Fig. 1D). These pectic layers ostensibly control wall thickness and allow for easy slippage between the cellulose-hemicellulose layers, which are thought to control wall extension.

There seems to be no definitive evidence at present favoring the tethered network model over the others. Recent work has yielded new evidence that a small amount of xyloglucan is indeed attached to pectic polysaccharides (23), thus reviving the model of Figure 1A. Whether this pectin-xyloglucan molecular functions to hold microfibrils together remains to be seen.

What these models have in common is the concept that cellulose microfibrils are coated with xyloglucan. The importance of cellulose microfibrils in wall structure was underscored by the discovery of an Arabidopsis mutant with a defect in a gene encoding a cellulose synthase (1). This mutant makes cellulose, but fails to assemble it correctly into a microfibril,

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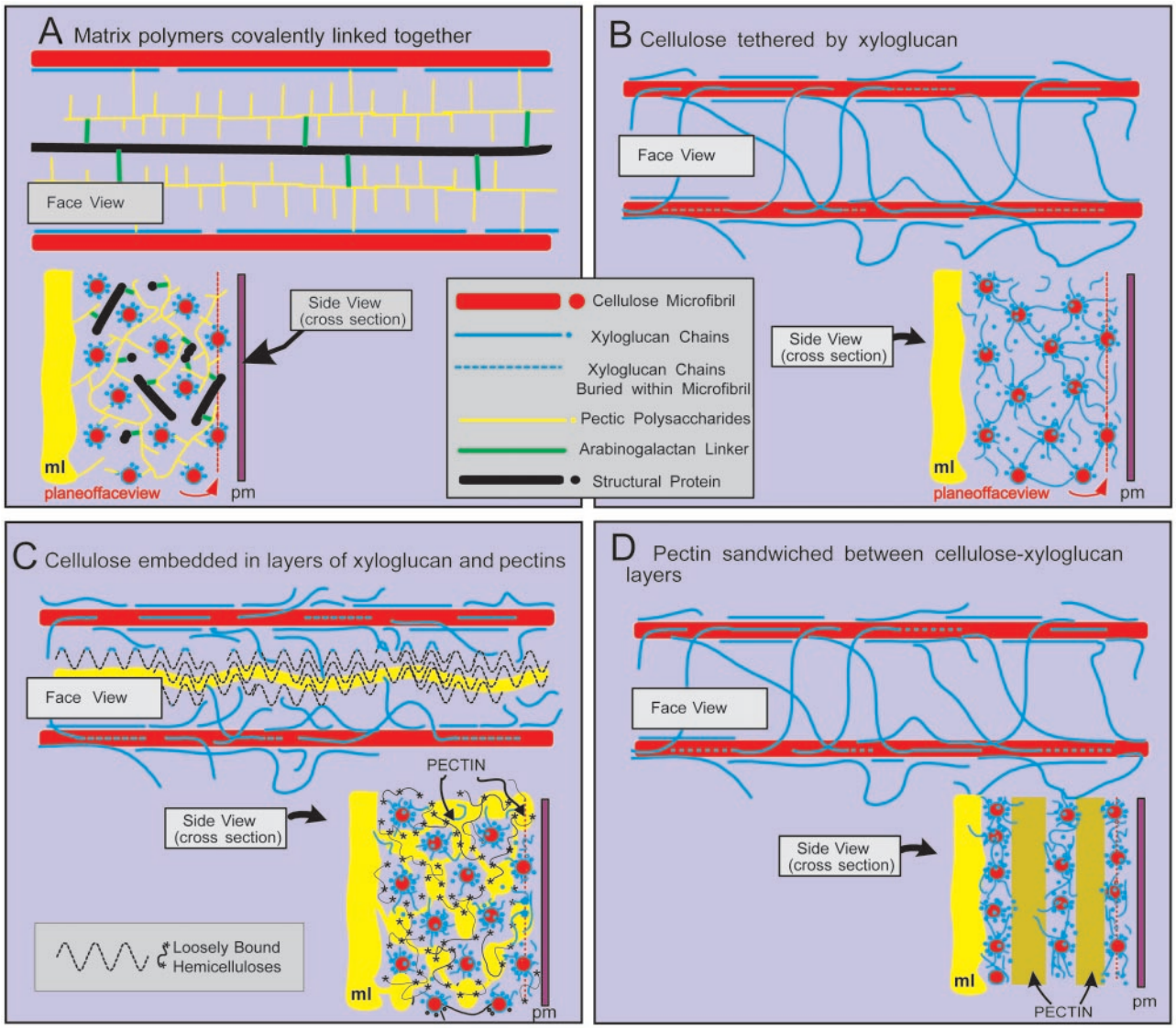


**Figure 1.** Alternative models of cell wall structure. A, The model of Keegstra et al. (10) in which the matrix polymers are all covalently linked to one another and anchor cellulose by hydrogen bonding to xyloglucans. B, The "tethered network" model of Hayashi (9) and Fry (6) in which single xyloglucan chains span the gap between microfibrils and tether them together. The pectic polysaccharides and structural proteins are not shown, but occupy the space between xyloglucan chains. C, The "multicoat" model of Talbott and Ray (21) in which cellulose is coated with successively looser layers of matrix polysaccharides. D, The "stratified" wall model of Ha et al. (8) in which strata of pectic polysaccharides separate cellulose-xyloglucan lamellae. pm, Plasma membrane; ml, middle lamella.

resulting in severe defects in wall structure and ordered growth. This discovery was also important as a confirmation of the landmark paper by Delmer and coworkers (17) identifying the first cellulose synthase gene in plants. We can anticipate that genes encoding the many glycosyl transferases required for synthesis of other wall polysaccharides will be identified soon (18).

## MATRIX METABOLISM AND ITS RELATION TO WALL LOOSENING

The wall contains many enzymes able to modify matrix polysaccharides (7). These include various endoglycanases that may cleave the backbone of matrix polysaccharides; glycosidases that may remove side chains, thus allowing greater interactions between polysaccharide backbones; transglycosylases that may cut polysaccharides and ligate them together; esterases that can remove methyl groups from pectins and cleave ester linkages between polysaccharide chains; and peroxidases that may form or break phenolic linkages in the wall.

These enzymes offer many possibilities for altering wall structure, and thereby modulating wall expansion. Here we run into a subtlety of what constitutes wall loosening. Wall hydrolytic enzymes may physically weaken the wall, yet not induce wall extension

(3); hence, they do not cause wall loosening by this definition. The crucial test is whether an enzyme is capable of inducing wall extension. I have suggested that enzymes with this ability be termed primary wall loosening agents, whereas other enzymes that have a less direct action be termed secondary wall loosening agents (i.e. they modify the wall to make it more responsive to the action of primary wall loosening agents [3]).

Twenty-five years ago, auxin-induced matrix hydrolysis attracted the greatest attention as a possible wall-loosening mechanism. Matrix polysaccharides exhibit substantial hydrolysis and turnover in growing tissues, yet most of this appears to be independent of extension growth. Labavitch and Ray (11) identified a xyloglucan fraction whose turnover is specifically accelerated by auxin in dicot tissues, whereas in grass coleoptiles Nevins and colleagues emphasized turnover of (1→3),(1→4) β-glucan (22). These metabolic changes are associated with increases in wall plasticity, i.e. as measured by stretching the wall in a tensile tester (12). Moreover, wall glucanases have also have been implicated as wall-loosening agents by Nevins, Masuda, and their colleagues on the basis of growth inhibition by antibodies and lectins that interfere with wall hydrolysis (22). Recent work has identified a novel endo-1,3;1,4-β-glucanase from maize coleoptiles that is associated with auxin-induced matrix hydrolysis (22). However, neither this nor other plant endoglucanases have been shown to induce extension of walls in vitro.

On the other hand, an important role for endoglucanase during wall assembly is indicated by an Arabidopsis mutant that is defective in a gene encoding a putative membrane-bound endoglucanase (15). Because this endoglucanase is not located in the wall, it probably does not function as a primary wall loosening enzyme; rather, it may process wall polysaccharides en route to wall assembly.

In the past decade xyloglucan endotransglycosylase (XET) emerged as another candidate for a wall-loosening enzyme (7, 16). XET is able to cut and ligate xyloglucan chains together, possibly allowing for a self-limiting extension of the wall. Despite the attractiveness of this idea, XET does not induce wall extension in isolated walls (3). XET may function to integrate newly secreted xyloglucans into the existing wall network (16).

## ACID GROWTH AND EXPANSINS

In the 1970s a large body of work established that plant cells enlarged faster when wall pH was reduced below approximately 5.5; this "acid-growth" behavior was also characteristic of isolated walls and so emerged the concept of a wall-loosening enzyme with a low pH optimum. It was proposed that the initial phase of auxin-induced growth involved a rapid acidification of the wall, stimulating one or more hypothetical wall-loosening enzymes (19). In the longer term auxin also stimulates wall synthesis, increases wall plasticity, and activates genes for wall enzymes and $H^+$-ATPases, all of which may be important factors for sustained auxin-induced growth.

Attempts in my laboratory to identify wall proteins with the ability to induce wall extension in vitro resulted in the isolation of two active proteins, later named expansins (14). These proteins mediate the acid-induced extension of cucumber hypocotyl walls (3). We subsequently identified a second family of expansins from grass pollen (5). Members of this family are referred to as β-expansins to distinguish them from the original family of expansins, now called α-expansins (see the expansin web site at www.bio.psu.edu/expansins/). The two kinds of expansins have similar physical effects on cell walls, but differ in their substrate preferences (4).

Expansins characteristically induce extension and stress relaxation of isolated cell walls, but they do not hydrolyze the major polysaccharides of the wall matrix (14), nor do they increase wall plasticity (S. Yuan and D. Cosgrove, unpublished data) or increase the average mobility of wall polysaccharides, as measured by nuclear magnetic resonance methods. Thus their physical effects on the cell wall are subtle.

The evidence for expansins as significant wall-loosening agents may be summarized as follows (4): (a) They induce extension of walls in vitro; (b) their application to living cells induces faster growth; (c) their genes are expressed at the right time and in the right place to function in growth control; and (d) reduction of expansin gene expression by antisense methods results in growth inhibition. Additional functions for expansin in fruit softening were suggested by Jocelyn Rose and Alan Bennett (20) who found high expression of an expansin gene during the last stages of fruit ripening.

The time course for expansin action is revealing, in that walls begin extending within seconds of protein addition, without the lag expected of a hydrolytic enzyme that acts by changing matrix viscosity. Expansin appears to act catalytically, rather than stoichiometrically (3). It can weaken artificial cellulosic composites such as paper and *Acetobacter* pellicles (25). Our current model for expansin action proposes that it induces slippage between load-bearing polysaccharides at a limited number of sites in the wall (3, 4). This might be at the cellulose-xyloglucan interface, but these interfaces may be too abundant for effective action by expansin; rarer sites within the wall seem more likely. What these are is still uncertain.

## A LOOK AHEAD

Although we have learned much in the past 25 years about the structure of the individual polymers that make up the cell wall (2), our ideas of how they

form a strong yet extensible network in the growing wall are still based on indirect evidence and biochemical hunches. More definitive results in the future may come from methods such as nuclear magnetic resonance, Fourier transform infrared spectroscopy, and atomic force microscopy, but the complexity of the wall presents daunting challenges for interpreting the results of these methods. Enzymes that cut specific sites in the wall (2) offer much potential for structural and mechanical analyses of wall structure. The expanding sequence databases and growing resources for reverse genetics offer additional approaches for testing the role of specific genes in wall structure and function.

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