

# Probing expansin action using cellulose/hemicellulose composites

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## Summary

Cellulose-based composite materials containing xyloglucans or mannan-based polysaccharides have been shown to possess organisational features with many characteristics similar to primary plant cell walls. We have tested the effects of a typical  $\alpha$ -expansin (CsExp1) on these composites using two different mechanical assays. We show that CsExp1 induces very rapid extension in composites containing tamarind xyloglucan under constant load. In contrast, expansin treatment had no effect in constant load extension assays using cellulose-only materials or in those carried out on composites containing glucomannan or galactomannan. We show that the effect of expansins is much smaller on composites made with short chain length xyloglucans than on those containing longer chains. In uniaxial extension tests we found that expansin could double the total extension (before failure) in xyloglucan composites and that the effects were again lower in composites containing shorter xyloglucans. We found no effect of expansin on uniaxial extensions with glucomannan or galactomannan. However, a significant effect of expansin on the uniaxial extension behaviour of cellulose-only material was observed. These experiments suggest that the target of CsExp1 in cell walls is probably the cellulose xyloglucan matrix, but that other (1-4)  $\beta$ -glucan to (1-4)  $\beta$ -glucan hydrogen bonded contacts can also serve as substrates.

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## Introduction

The primary cell wall is believed to comprise of two co-extensive, interpenetrating networks: a cellulose/hemicellulose network embedded in a pectin matrix, with structural proteins potentially forming a third domain (Carpita and Gibeaut, 1993; McCann and Roberts, 1992; Talbott and Ray, 1992). During expansion, the cell may enlarge to as much as 100 times its original volume. Both wall thickness and microfibril spacing are conserved so the expansion process requires extensive architectural rearrangement of existing material, coupled with the synthesis of new cell wall components (Carpita and Gibeaut, 1993). The loosening of the wall which is necessary for turgor-driven expansion to occur is generally believed to be a biochemical process (Cosgrove, 1997), catalysed by wall-loosening enzymes.

The cellulose/hemicellulose network is thought to be the major load-bearing component in primary cell walls and is therefore likely to be the main target for wall-loosening enzymes. In the Type I cell walls of dicotyledonous and non-graminaceous monocotyledonous plants, the major

hemicellulose is xyloglucan. Xyloglucan is believed to coat cellulose and form cross-links between adjacent microfibrils, creating an aligned, cross-linked network (Carpita and Gibeaut, 1993; McCann and Roberts, 1992; McCann *et al.*, 1990). Other hemicelluloses, such as mannan-based polysaccharides, are also found in Type I cell walls (Bacic *et al.*, 1988); these are generally present in low amounts, but have recently been shown to be capable of binding and cross-linking cellulose (Whitney *et al.*, 1998).

The identity of enzymes which catalyse wall relaxation and yielding during cell expansion remained elusive until the discovery of two proteins, isolated from cucumber hypocotyls, which restored extension activity to heat-inactivated walls (McQueen-Mason *et al.*, 1992). These 25 kDa proteins, subsequently named expansins, were shown to account for the majority of acid-induced extension in cucumber hypocotyls. Expansins are a novel class of cell wall proteins and have been identified in a range of monocotyledonous and dicotyledonous species and plant organs. Expansins from such diverse sources as ripening

tomato fruits (Rose *et al.*, 1997) and seedlings of *Arabidopsis* and rice (Shcherban *et al.*, 1995) are shown to have 70–90% homology at the amino acid level (Shieh and Cosgrove, 1998).

Thus far, expansin action has mostly been characterised using plant tissues and the molecular target for expansin action is still obscure. Expansins have no detectable exoglycanase (McQueen-Mason *et al.*, 1992), endoglycanase (McQueen-Mason *et al.*, 1992; McQueen-Mason and Cosgrove, 1995) or xyloglucan endotransglycosylase (McQueen-Mason *et al.*, 1993) activity. Expansins were shown to weaken pieces of filter without hydrolysing cellulose fibres, suggesting that hydrogen-bonded interactions between the fibres were disrupted (McQueen-Mason and Cosgrove, 1994). Indirect evidence was also presented that expansins may disrupt hydrogen bonding during extension in hypocotyl segments. Binding experiments showed that expansins bound relatively weakly to pure cellulose, but more strongly to cellulose coated with hemicelluloses, indicating that expansins bind at the interface between cellulose microfibrils and matrix polysaccharides. Taken together these data suggest that expansins act by disrupting hydrogen bonding between cellulose microfibrils and hemicelluloses leading to wall loosening and extension. This hypothesis is, however, based largely on indirect evidence, and the molecular mechanism of expansin action remains uncertain.

Due to their complexity, plant tissues are not ideal materials for assaying and characterising expansin activity. Extension assays for expansin activity are typically carried out on frozen-thawed, heat-treated pieces of plant organs such as cucumber hypocotyls (McQueen-Mason *et al.*, 1992). Such material is highly heterogeneous in its cellular, as well as its cell wall, composition. For this reason, we have looked at expansin action on simpler materials with a defined composition which resemble some aspects of cell wall architecture. *Acetobacter xylinus* (ATCC 53524) synthesises highly crystalline cellulose as an extracellular polysaccharide. In the presence of tamarind xyloglucan, two component structures are formed with molecular organisational and ultrastructural features in common with those of similar networks from cell walls (Whitney *et al.*, 1995). Similar networks can also be formed with other hemicelluloses such as mannan-based polysaccharides (Whitney *et al.*, 1998).

There are two advantages to using *Acetobacter* cellulose composites for examining expansin action. One is that the networks are simple, molecularly defined, two component systems, with none of the complexity of plant tissues. Secondly, the networks are synthesised as continuous cm-sized sheets suitable for mechanical testing (Whitney *et al.*, 1999). In contrast, plant materials used in extension measurements are discontinuous as they are composed of many cell walls held to one another by middle lamellae.

In the work presented here, we examine the effects of expansins on the mechanical properties of a range of composite materials in order to determine the molecular targets for expansin action.

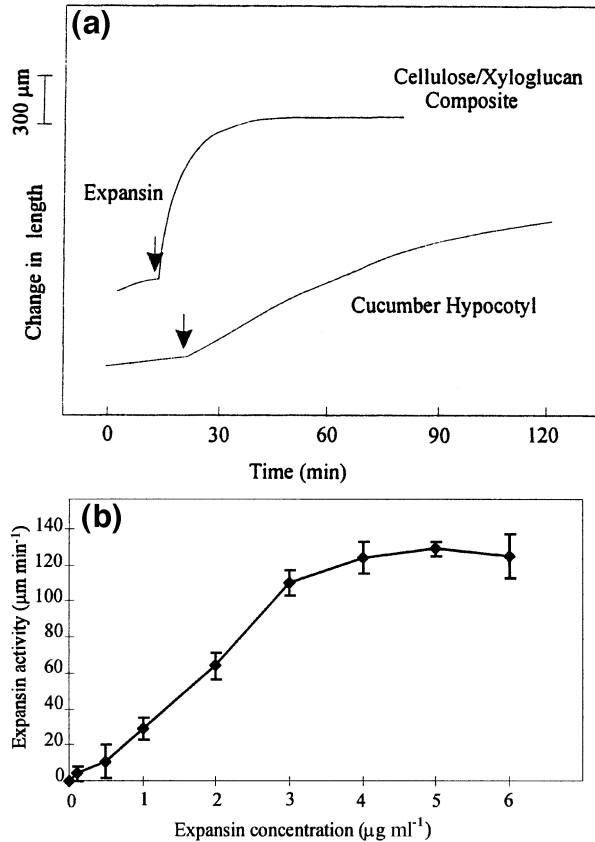
## Results

### *Expansin induces the extension of xyloglucan/cellulose composites*

CsExp1 was originally isolated from etiolated cucumber hypocotyl cell walls, and has been characterised by its ability to restore extension (as assayed in an extensometer) to heat-inactivated walls from this tissue (McQueen-Mason *et al.*, 1992). It is difficult and expensive to isolate sufficient native CsExp1 from cucumber hypocotyls for extensive biochemical work. Therefore, in the work presented here we have used purified recombinant CsExp1 produced in transgenic tomato which have been shown to have similar activity to the native form (F.S. Rochange and S.J. McQueen-Mason, unpublished results).

Composites formed with high molecular weight tamarind xyloglucan, which have molecular and ultrastructural features in common with Type I cell walls (Whitney *et al.*, 1995), show a significant expansin response. Typical extension curves of expansin-treated tamarind xyloglucan/cellulose composite and a piece of inactivated cucumber hypocotyl are compared in Figure 1(a). Clearly, the expansin initially has a more dramatic effect on the composite than it does on the hypocotyl section and the shapes of the extension curves are somewhat different. In the composite material most of the extension occurs within 10–15 min of expansin application with averaged rates of extension of about  $90 \mu\text{m min}^{-1}$  (almost  $3\% \text{ min}^{-1}$ ) during the first 10 min. In contrast, expansin-induced extension in the hypocotyl material is close to an order of magnitude slower in the early minutes after expansin treatment, at about  $10 \mu\text{m min}^{-1}$  (close to  $0.3\% \text{ min}^{-1}$ ). In both cases the rates of extension decay with time, but this deceleration is more marked in the composite material than in the hypocotyl section. After about 15 min of expansin-induced extension the composite has returned almost to its initial extension rate and soon approaches zero. In the hypocotyl section, however, extension continues at a steady, slowly decaying rate for a number of hours and, if left long enough, the total percentage extension is of a similar magnitude to that seen in the composite material. Figure 1(b) shows the effect of different concentrations of expansin on the extension of the xyloglucan/cellulose composite. Expansin activity is detectable with as little as  $100 \text{ ng mL}^{-1}$  of expansin and reaches saturating levels at about  $3.5 \mu\text{g mL}^{-1}$ .

Figure 2 compares the effect of expansin on the extension of five different materials produced by *Acetobacter*



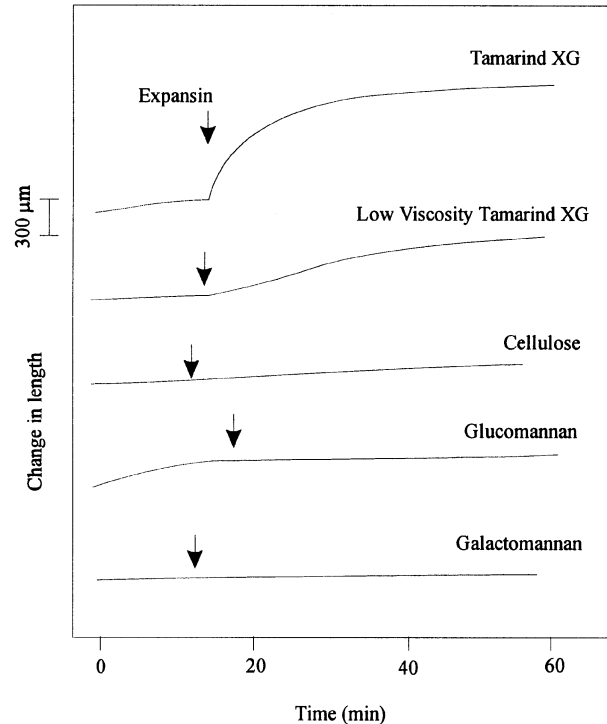
**Figure 1.** Extension under constant load of various materials in the presence of recombinant CsExp1.

(a) Extension of a 2 mm wide strip of cellulose/xyloglucan composite formed by *Acetobacter* (upper trace) and of a heat-inactivated cucumber hypocotyl section (lower trace).

(b) Concentration dependence of expansin activity on xyloglucan/cellulose composite. All materials were initially extended in the presence of 50 mM sodium acetate, pH 4.5 to establish a base line of extension without added expansin. Once this initial extension reached a steady rate, the bathing solution was exchanged for 100 µl of the same buffered solution containing 1 µg ml<sup>-1</sup> of recombinant expansin. The imposed weights for each extension were 11 g for the cellulose/xyloglucan composite, 20 g for the cucumber hypocotyl, 35 g for the cellulose only pellicle, and 20 g for the Whatman 3MM. Representative traces of six replicate measurements are presented.

fermentation. The first trace again shows the dramatic effect of expansin on the cellulose/tamarind xyloglucan composite. Because tamarind xyloglucan polymers are generally much longer than those found in a typical growing plant cell wall, we investigated the effect of making composites with a low viscosity fraction of tamarind xyloglucan. This xyloglucan has an average molecular mass more similar to that seen in growing walls (30–60 000 kDa compared to greater than 500 000 kDa for the high molecular mass xyloglucan). The second trace shows that composite made with this low viscosity xyloglucan has a much smaller response to expansin.

In contrast to these composites, little or no expansin effect is seen in the extension of cellulose-only material. It

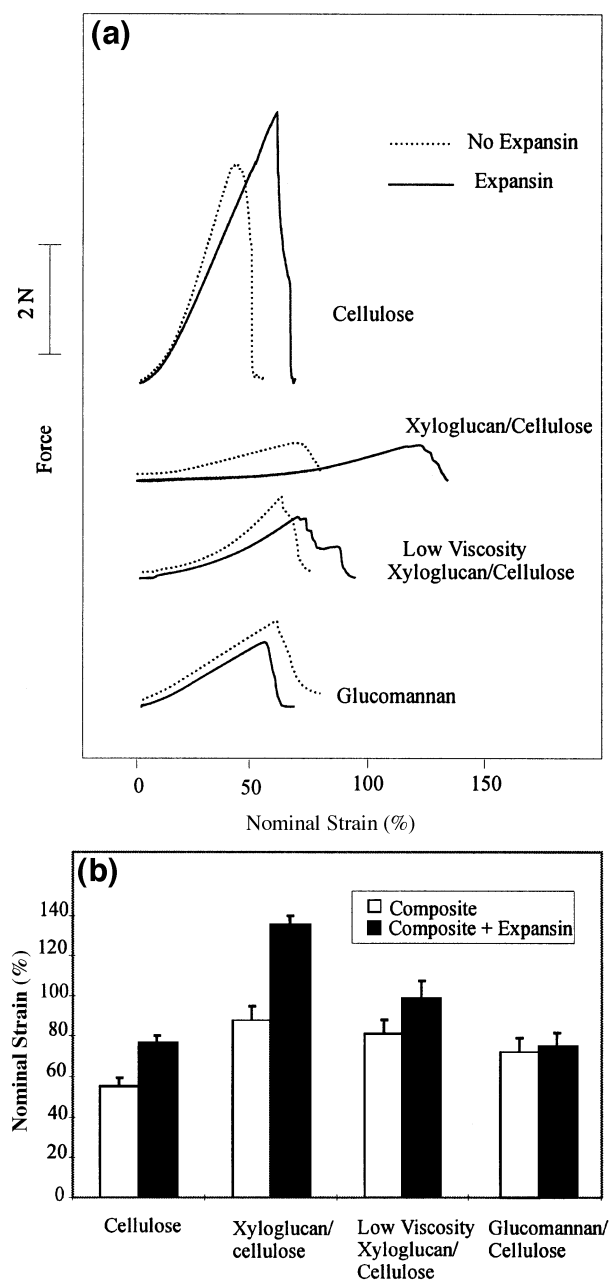


**Figure 2.** Effects of expansin on the extension of *Acetobacter*-made matrices under constant load.

Treatments were essentially the same as those described in Figure 1. Materials used were *Acetobacter* composites made in the presence of high molecular mass tamarind xyloglucan (Tamarind XG), low viscosity tamarind xyloglucan, cellulose-only, glucomannan or galactomannan. All strips were cut at 2 mm wide except for the cellulose-only which was cut 1 mm wide. The imposed load for the composites was 11 g in all cases except for the cellulose-only on which 35 g was used. Representative traces for all treatments are presented. Extension measurements were repeated six times. Expansin-induced extension was calculated as the averaged rate of extension in the 5 min period after expansin addition minus the averaged rate in the 5 min prior to expansin addition. Average extension rates and standard deviations were as follows: xyloglucan/cellulose 96.3 µm min<sup>-1</sup>, S=21.8; low viscosity xyloglucan/cellulose 24.8 µm min<sup>-1</sup>, S=6.7; cellulose only 0.4 µm min<sup>-1</sup>, S=0.7, glucomannan–5.3 µm min<sup>-1</sup>, S=0.2; galactomannan 0.5 µm min<sup>-1</sup>, S=0.3.

should be noted, however, that this material is a lot stiffer than the xyloglucan composites and although a narrower strip of material was used with more than three times the extensive force (35 g was applied for cellulose compared to 11 g for the xyloglucan composite), little or no extension was apparent before or after expansin application. In contrast, the xyloglucan/cellulose composite is much less stiff and shows substantial extension even before expansin addition.

We also assayed the effects of expansin on composites made with mannans (Figure 2). Glucomannans are hemicellulosic components of most Type I cell walls, often present as galactoglucomannans (Meier and Reid, 1982). Like xyloglucan, both glucomannan and a range of galactomannans bind and cross-link cellulose in the *Acetobacter* system and also perturb cellulose organisa-



**Figure 3.** Effects of expansin on the uniaxial extension behaviour of *Acetobacter*-made matrices.

(a) The same materials as used in Figure 2 were subjected to uniaxial extension in an Instron tensile test device. Materials were either preincubated in 50 mM sodium acetate, pH 4.5 or in the same solution containing  $5 \mu\text{g ml}^{-1}$  expansin. Eight replicate measurements were made for each treatment and a representative trace for each is presented.

(b) Effect of expansin on the extension of the various matrices presented above. Average extension values and standard error for each experiment are presented ( $n=8$ ). Student's *T*-tests were performed on each pair of data sets to determine the significance of apparent differences. *T*-values for the effect of expansin treatments were cellulose-only 0.021, xyloglucan/cellulose  $9.9 \times 10^{-5}$ , low viscosity xyloglucan/cellulose 0.085, glucomannan/cellulose 0.35. The effect of expansin on cellulose-only is significant within a confidence limit of 0.05, and highly significant on the xyloglucan/cellulose composite. The effect on low viscosity xyloglucan/cellulose and glucomannan is not significant within this confidence limit. Overall, experiments were repeated at least twice with similar results.

tion, indicative of an intimate molecular interaction (Whitney *et al.*, 1998). Despite similarities of network architecture and molecular features with xyloglucan-based material, neither glucomannan nor galactomannan composites respond to the application of expansin. Both the glucomannan- and galactomannan-containing composites are much less stiff than the cellulose-only material and showed similar extension behaviour in the absence of expansins to that of the xyloglucan-containing composite. Despite this mechanical similarity to the xyloglucan composite, neither of these mannan-containing composites showed any response to expansin application, indicating that CsExp1 action is specific for the xyloglucan/cellulose composite.

In order to investigate the relationship between inherent stiffness of the materials and their sensitivity to expansins, we carried out a series of uniaxial extension measurements. In these measurements a strip of material is extended at a constant rate and the resulting build-up of mechanical stress recorded until the material fails, giving a measure of the mechanical properties of the material over a spectrum of applied loads. This contrasts with the data in Figures 1 and 2 which are produced by the extension of the materials under a constant applied force. Figure 3(a) compares the uniaxial extension behaviour of the different composites with and without added expansin. The first two traces show typical extensions for the cellulose-only material. Although no effect of expansin was detectable in the constant-load extension assays, a clear effect of expansin is seen in the uniaxial extensions. Figure 3(b) confirms that expansin treatment led to a significant, almost 30%, increase in extension before breakage of this material. We propose that the reason little or no effect of expansin is seen in constant load extensions of this material is due to its inherent stiffness and strength, and that the force applied for these experiments was insufficient to produce extension.

The second set of traces in Figure 3(a) shows the uniaxial extension of the xyloglucan/cellulose composite and reveals that this material is far less stiff, and more extensible, than the cellulose-only material and that the effect of expansins on mechanical properties is even greater. The average extension of this material is around 35% but is more than doubled to around 75% in the presence of expansin (Figure 3b). The third set of traces in Figure 3(a) show that the composite made with low molecular mass xyloglucan had a similar inherent extensibility to the composite made with high molecular mass material, but that the effect of expansin was much smaller in this material. This agrees with the data obtained from constant-load extensions which also showed a reduced effect of expansins on this composite.

Uniaxial extension measurements with the glucomannan/cellulose composite (Figure 3a) revealed a similar

force/deformation relationship to that seen in the xyloglucan/cellulose composite. This material is much less stiff and more extensible than the cellulose only material. However, unlike the xyloglucan/cellulose, the glucomannan/cellulose composite shows no significant (Figure 3b) effect of expansin on its extension curve. This agrees with the lack of expansin effect seen in the constant load measurements presented in Figure 2.

## Discussion

### *Expansin acts on cellulose-hemicellulose composites in vitro*

The composite material formed with high molecular weight tamarind xyloglucan showed sufficient similarities with native networks in terms of molecular features and ultrastructure that it was proposed as a simplified model system for plant cell walls (Whitney *et al.*, 1995). Despite some differences in the nature of the expansin response between these materials and plant tissues, the observation that they are expansin-sensitive provides additional validation that these composites reflect certain aspects of primary plant cell walls. Indeed, we have found that the cellulose/xyloglucan composite is a far more sensitive material for assays of expansin activity than any of the plant materials we have tested. Using the composite it is possible to detect activity at almost a 10-fold lower concentration than using inactivated cucumber hypocotyls (data not shown). There are additional attractive features to using the composite for expansin assays. First, the activity measurements on the composite material are less variable than on hypocotyl walls which means a smaller number of replicate measurements need to be made. There are several reasons why this may be so. The hypocotyl sections come from individual seedlings which themselves will have varying growth rates which, presumably, will be reflected by wall extensibility. Secondly, there is a gradient of growth rate and wall extensibility along the hypocotyl section (McQueen-Mason, 1995) which means that differences in the positions of the clamps on a section will lead to differences in the extensibility of the material between them.

Another advantage of the composites is that they are continuous well-defined materials. In contrast, the hypocotyl sections are of a largely undefined and heterogeneous nature. We still poorly understand the structure and function of plant cell walls, and the material itself is composed of a range of different cell types (including epidermal, cortical and vascular cells), the walls of which differ not only in their thickness but also, most probably, in their composition. In addition, it is not clear what is happening at a cellular or molecular level during the extension of a piece of plant tissue in an extensometer: is it

the walls of all the cells that are extending together in a coordinated manner, or does extension also involve the sliding of cells relative to one another in the tissue? The relative simplicity and homogeneity of *Acetobacter*-made composites opens up the possibility of a molecular definition of expansin action which would be far more difficult to achieve using plant tissues as substrates.

### *Expansins show effects on cellulose-only material in uniaxial but not in constant load extensions*

We found that there were no measurable effects of expansins on cellulose-only material in extensometer assays, but that significant effects on total extension were apparent in uniaxial extensions. This discrepancy is probably due to the relatively small forces used in constant load extensions compared to the inherent stiffness of this material. These data contrast with results reported on the effects of expansins on pure cellulose filter or chromatography paper. McQueen-Mason and Cosgrove (1994) showed that expansins could induce low levels of extension (followed by breakage) in Whatman filter paper. Similarly, Bolam *et al.* (1998) showed that expansin treatment weakened Whatman 3MM paper by more than 30% in uniaxial extensions. Such apparent discrepancies may be explained by the very different structure of these materials. Papers such as 3MM are composed of randomly aligned cotton fibres which are in fact each a wall from a single fibre cell, rather than the microfibrils that make up the pellicle. This means that the structural units of paper are several orders of magnitude greater than those that make up the pellicle. Hence, the numbers of fibre per unit amount of paper are relatively small (as compared to the pellicle), and the contacts and entanglements between fibres are also relatively short. This makes for a weaker material in which a potentially small effect of expansins can have a relatively large overall effect. In contrast, the pellicle is composed of cellulose microfibrils which are relatively long and form a dense, highly entangled mat (Whitney *et al.*, 1995) leading to a more cohesive and strong material. It is interesting to note that the relative sparsity of strength-determining interactions in paper shares some similarity with the domain model (Whitney *et al.*, 1999) in which the cellulose/xyloglucan composite has fewer mechanically important interactions than pure cellulose pellicle.

### *Mannan/cellulose composites are insensitive to expansins*

There was no detectable effect of expansin on mannan-containing composites in either uniaxial or constant load extensions. This finding is important in clarifying our understanding of expansin action. The composites formed

with gluco- or galactomannans show structural and mechanical features similar to those formed with the tamarind xyloglucan (Whitney *et al.*, 1998). Microscopic examination shows very similar alignments of the cellulose microfibrils and also the presence of interfibril crosslinks. In addition, the uniaxial extension profiles show that, like the xyloglucan/cellulose, the mannan/cellulose composites are more extensible than the cellulose-only material. The absence of any expansin effects on these materials, taken in context with the other data presented here, strongly suggests that CsExp1 action is specific for (1-4)  $\beta$ -glucan-based polymers. Initial studies also indicate that this expansin exhibits no effects on the mechanical properties on similar composites made with wheat arabinoxylan (S.E.C. Whitney *et al.*, unpublished results).

#### *The role of entanglements in mechanical properties*

An examination of the shapes of the uniaxial extension curves may be informative to our understanding of the mechanical properties of these materials. The force/deformation curve for cellulose-only material remains reasonably linear as the material stretches, and is also much steeper than that seen in the composites. We suggest that this stiffness reflects the entanglements of the cellulose fibrils as revealed by microscopic studies (Whitney *et al.*, 1995). As suggested by Whitney *et al.* (1999), the increase in stress in this material during extension could result from entanglements being pulled to their limit of extension, rather like a knot being tightened. As a material extends, entanglements between fibres and polymers will inevitably be tightened. Once this happens, that particular domain in the material will have reached its limit of extension. At this point other domains can keep sliding until their limit of extension is reached. The more rapidly these 'terminal entanglements' accrue, the stiffer the material. As more limits of entanglement are reached so stress builds up in the material, until eventually a cataclysmic failure occurs. Expansins allow the cellulose-only material to extend to a greater extent. We suggest that this is because expansins lubricate glucan-glucan interactions allowing (in some instances) entangled polymers (or fibrils) to slide relative to one another rather than becoming tightened. This means that the material can extend further before entanglements become terminally stretched and failure occurs.

In the composite made with tamarind xyloglucans, the fibrils are more locally aligned and less entangled (Whitney *et al.*, 1995). Despite being highly crosslinked, the composite is weaker than cellulose alone, indicating that a long (cf. molecular) distance scale mechanism is operative. It has been suggested previously (Whitney *et al.*, 1999) that there are domains of cross-linked cellulose which are internally strong but only weakly connected to

adjacent domains. These domains are envisaged to be capable of sliding past each other resulting in a weaker tensile behaviour: no apparent breakage of xyloglucan crosslinks is observed microscopically after tensile extension to failure (Whitney *et al.*, 1999). Cucumber expansin is envisaged to facilitate the relative movement of cellulose/xyloglucan domains by molecular lubrication of domain interactions. Such a mechanism is effective provided the 'knots' defining the limit of extension are parallel (Figure 6B in Whitney *et al.*, 1999) but not when they are 'looped' (Figure 6A in Whitney *et al.*, 1999). In constant load extensions, expansin induces about 30–40% increases in length of cellulose/xyloglucan composite after which extension approaches zero. We found that the addition of more expansin at this point did not induce further extension, and suggest that at this point 'knots' restraining extension are of the 'looped' kind and not sensitive to expansin.

#### *Expansin is less effective on composites made with short xyloglucans than those made with long xyloglucans*

Although uniaxial extensions showed that composites made with short chain xyloglucan behaved similarly to those made with long chain xyloglucan, the effect of expansin on these composites in constant-load extensions and uniaxial extensions was substantially lower. Microscopically, composites made with lower molecular weight xg show fewer cross-links than those at a higher molecular weight. This is interpreted as indicating that domains of cross-linked cellulose are less well-defined for lower molecular weights resulting in extension characteristics (e.g. tensile stiffness [slope of force/deformation prior to break] and expansin sensitivity) intermediate between cellulose and composites with high molecular weight xyloglucan. We suggest that the reduced cross-linking extent results in less expansin sensitivity because of the reduced definition of cross-linked domains, the surface of which are hypothesised to be the site of expansin action.

It is pertinent to this discussion to point out that a clear difference between the *Acetobacter*-made composites and a typical primary cell wall is that of scale. If one compares the micrographic images of onion parenchyma walls published by McCann *et al.* (1990) with that of the xyloglucan composites published by Whitney *et al.* (1995), it is clear that although xyloglucan crosslink lengths are similar, the spacing between (non-crosslinked) microfibrils in the composite is almost an order of magnitude greater than that seen in the cell wall. The two different xyloglucan fractions used in this study differ in size by a similar factor. For this reason it may be that only high molecular mass polymers such as the tamarind xyloglucan (or the gluco- and galactomannans) used in

this study are long enough to provide substantial numbers of crosslinks between the fibrils in *Acetobacter*-made matrices. In plant cell walls it may be that shorter xyloglucans can fulfil this crosslinking role, and thus also serve as substrates for expansins.

In summary, we have shown that xyloglucan/cellulose composites produced by *Acetobacter* cultures are very sensitive substrates for expansins. This further reinforces the suggestion that these composites reflect important aspects of primary plant cell wall structure (Whitney *et al.*, 1995). Expansin was active on composites containing xyloglucan but not on those made with mannan polymers, indicating that the cellulose/xyloglucan matrix is indeed the site of expansin action, and inferring that this may be the main determinant of mechanical properties in growing walls. Our data show that interactions between 1-4  $\beta$ -linked glucans are the site of action of cucumber expansin with xyloglucan/cellulose systems being the most sensitive. We speculate that expansins catalyse the molecular lubrication of glucan/glucan interactions. As such interactions are numerous in the systems studied, the effective catalysis of extension at very low expansin levels suggests a site of action that differs in molecular detail from the bulk of the system. We speculate that molecular interactions between glucans which limit extension involve high energy polysaccharide chain conformations and/or H-bonding, and that these local high energy environments are the site of expansin catalysis. As *Acetobacter* cellulose is a substrate for expansin this suggests interglucan H-bonding (possibly via water) as a primary site. The greater sensitivity of xyloglucan composites, however, opens up the possibility that the conformational freedom of xyloglucan (Levy *et al.*, 1997; Whitney *et al.*, 1995) may result in a local high energy conformation as a more effective site of expansin action. Further progress on these molecular details of action require 3D information on expansin molecules from, for example crystallography or NMR spectroscopy. If, as suggested here, in the cell wall expansin action is also proportional to xyloglucan chain length, then the interactions between expansins and xyloglucan modifying enzymes such as XETs and endo-glucanases may be highly important in the modulation of wall mechanical properties. Finally, we emphasise the point that these data were obtained using a single expansin isoform, and it remains to be demonstrated whether the xyloglucan specificity shown here is true for all expansins or not.

## Experimental procedures

### Preparation of composite materials

*Acetobacter xylinus* cultures were incubated in Hestrin Schramm medium (Hestrin and Schramm, 1954) in the presence of 2% glucose only (controls) or with 2% glucose plus 0.5% tamarind xyloglucan (Dainippon Pharmaceutical Co., Osaka, Japan) pur-

ified as described by Gidley *et al.* (1991) or 0.5% Konjac glucomannan (purified from Konyaku flour as described by Whitney *et al.*, 1998) or 0.5% locust bean gum galactomannan (RL 200, Meyhall, Switzerland) purified from the commercial material as described by Whitney *et al.* (1998). Incubations were performed at 30°C for 72 h under static conditions. After 72 h, cellulosic pellicles were harvested, washed extensively in deionised water and stored in 0.02% NaN<sub>3</sub> at 1°C until use.

### Purification of expansin

Recombinant CsExp1 was produced in transgenic tomato plants expressing the *CsExp1* coding sequence (Genbank accession number U30460) under control of the Cauliflower Mosaic Virus 35S promoter (Rochange and McQueen-Mason, unpublished data), and purified by a combination of precipitation and cation exchange chromatography. Briefly, 250 g of frozen transgenic tomato plant was homogenised in a food blender (Kenwood BL350) for 1 min at full speed, with a double volume (with reference to fresh weight) of 25 mM HEPES, 3 mM sodium metabisulfite, 2 mM dithiothreitol (DTT), 1 mM EDTA, pH 6.8, 1% polyvinylpyrrolidone 40, 0.1% Triton X100. Walls were collected from the homogenate on a 100 micron nylon mesh, resuspended in the same volume of the same buffer, stirred for 1 min and again collected on the mesh. Walls were then rinsed three times in cold distilled water to remove the detergent. Washed and rinsed walls were then extracted in a volume equal to initial fresh weight 25 mM HEPES, 3 mM sodium metabisulfite, 2 mM dithiothreitol (DTT), 1 mM EDTA, 1 M NaCl, pH 6.8 for 1 h at 22°C. Extracted walls were retained on the nylon mesh and discarded. Proteins from the eluate from the walls were precipitated by the addition of 390 g l<sup>-1</sup> of ammonium sulfate, and pelleted by centrifugation at 10 000 g for 10 min at 4°C. Pelleted protein was resuspended in 15 mM MES, pH 6.5 and desalted into the same buffer on a small column of Sephadex G-25 (Pharmacia Biotech, St Albans, UK). Desalted protein was separated on an XK 16/20 column with a 10 cm bed height of SP Sepharose Fast Flow (Pharmacia Biotech) in 15 mM MES, pH 6.5 with a linear gradient from 0 to 400 mM NaCl at a flow rate of 3 ml per min and a total volume of 200 ml. Protein fractions were corrected to pH 4.5 by the addition of 1 M acetic acid, prior to expansin assays. Protein concentrations were estimated by densitometric comparison with protein standards on Coomassie-stained SDS-PAGE gels using an IS1000 Digital Imaging System (Alpha Innotech Corporation).

### Extensometer assays

*Acetobacter* pellicles were cut into strips approximately 2 mm (cellulose/XG) or 1 mm (cellulose) wide. Strips were pressed between microscope slides which were covered with a layer of paper tissue (Professional Wipes, Kleenex) under a weight of 500 g for 5 min to remove excess water and clamped in a custom-made extensometer, essentially as described by Cosgrove (1989), with about 3 mm of material between the clamps, while bathed in 100  $\mu$ l of 50 mM sodium acetate, pH 4.5. Extension under a constant applied load was continuously monitored by a micro-computer. Once a stable base rate of extension was established (after 10–20 min), the bathing solution was exchanged with one containing expansin with the pH adjusted to 4.5. Heat-treated cucumber hypocotyl segments were prepared for extension assays essentially as described by McQueen-Mason *et al.* (1992), and extended under an applied force of 20 g.

*Uniaxial tensile testing*

Rectangular strips of material were cut using a razorblade of typical geometry 15 × 3 × 1 mm. The two ends were placed directly between vice grips in an Instron 91–51–17 M extensometer (Instron SATEC Systems, Grove City, PA, USA) and the grips moved apart at a constant speed of 10 mm min<sup>-1</sup>. A 10 N load beam was used to record the force required for extension as a function of time. For expansin treatment, strips of material were drained on pieces of paper towel to remove excess fluid, prior to being incubated in a solution containing 5 µg ml<sup>-1</sup> expansin in 50 mM sodium acetate, pH 4.5 for 10 min and stored temporarily on ice prior to extension.

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