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Plant cell wall engineering: applications in biofuel production and improved human health

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Plant cell walls consist largely of cellulose, non-cellulosic polysaccharides and lignin. Concerted attempts are underway to convert wall polysaccharides from crop plant residues into renewable transport fuels and other valuable products, and to exploit the dietary benefits of cereal grain wall polysaccharides in human health. Attempts to improve plant performance for these applications have involved the manipulation of the levels and structures of wall components. Some successes in altering non-cellulosic polysaccharides has been achieved, but it would appear that drastic changes in cellulose are more difficult to engineer. Nevertheless, future prospects for both genetically modified (GM) and non-GM technologies to modify plant cell wall composition and structure remain bright, and will undoubtedly find applications beyond the current focus on human health and biofuel production.

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Introduction

The cellulose and non-cellulosic polysaccharides of plant cell walls represent the largest source of renewable carbohydrate on Earth [1] and, given the potentially huge mass of fermentable sugars embodied within these polysaccharides, it is hardly surprising that cell walls and their residues are attracting considerable interest for the production of renewable liquid transport fuels. In addition, plant cell wall polysaccharides are increasingly recognized as important constituents of dietary fibre in cereal grains and therefore of immense value in reducing the risk of burgeoning human diseases such as type II diabetes, colorectal cancer, cardiovascular disease and inflammatory bowel diseases [2,3••].

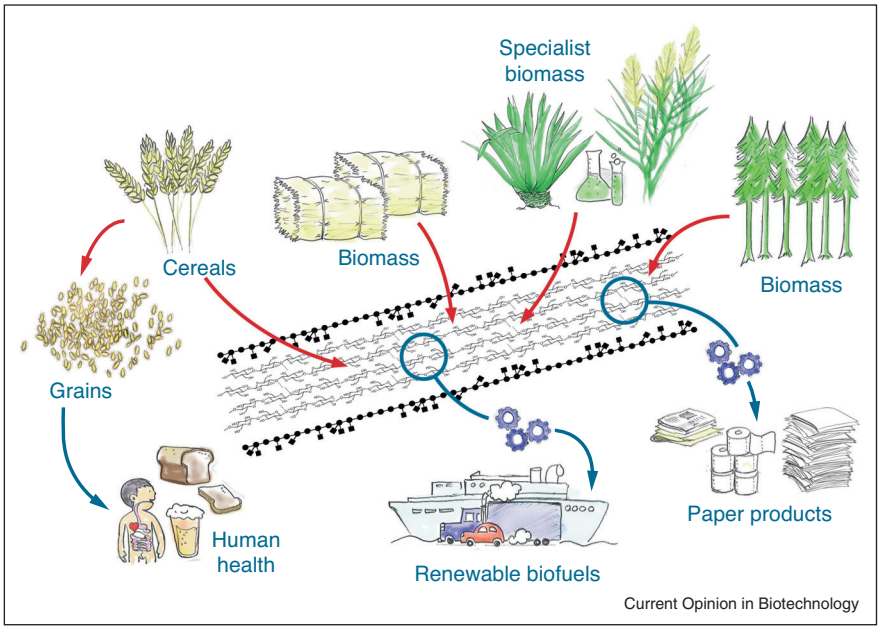
Engineering the relative amounts and chemical structures of wall components has the potential to dramatically improve the yields of biofuels from plant residues and to enhance the health benefits of cereal grains, and has been achieved through standard genetically modified (GM) procedures and through non-GM methods such as random mutagenesis and screening of natural variation. Here we will focus on the engineering of wall polysaccharides rather than lignin, given the extensive information on the genes that mediate lignin biosynthesis and the effects of manipulating expression levels of these genes [4]. In many cases the experiments described below were undertaken primarily to define the functions of candidate genes that had been implicated in the synthesis of particular wall polysaccharides, but the results have almost invariably revealed opportunities for engineering cell walls to address potentially valuable biotechnological applications (Figure 1).

Cellulose

Cellulose consists of linear chains of (1,4)-β-linked glucosyl residues that are synthesized at the plasma membrane and are believed to aggregate into highly insoluble microfibrils that are often viewed as reinforcing rods in the cell wall composite. New evidence is emerging to support a role for bundles of microfibrils in wall structure [5,6]. Cellulose synthase (CesA) enzymes are centrally involved in the process and form multi-enzyme transmembrane complexes that are embedded in the plasma membrane. Over the last decade it has been widely held that these complexes correspond to hexagonal terminal 'rosettes' that are clearly visible in the plasma membrane and that each complex most probably consists of 36 individual cellulose synthase enzymes that together synthesize a microfibril containing 36 parallel cellulose molecules. However, using a combination of spectroscopic and diffraction techniques, Thomas *et al.* [7••] recently provided compelling evidence that the microfibrils of collenchyma cell walls from celery (*Apium graveolens*) are 2.9–3.0 nm in diameter and are most likely to consist of 24 individual cellulose chains arranged in rectangular structures containing eight sheets of three chains each. On this basis, our 36-chain model for the cellulose synthase complex needs to be re-examined.

It was originally suggested that three different cellulose synthase isoenzymes were present in each complex and that a different set of three isoenzymes directed cellulose synthesis in primary and secondary walls. Other groups have suggested that four cellulose synthase isoforms are required in elongating cotton fibres for high

Figure 1



Engineering cell walls for human health and industrial applications. The cellulosic microfibrils and associated non-cellulosic polysaccharides of plant cell walls make up a high proportion of biomass sources such as wheat straw and timber. In the biofuels area, increased understanding of wall architecture, the fine structures of constituent polysaccharides, and the interactions between wall polysaccharides will facilitate the development of more efficient biomass deconstruction and conversion technologies. In this context, one wonders if current pre-treatments and enzymic conversion technologies for ethanol production, which are marginally viable at best and require tuning for individual feedstocks, might be replaced by 'feedstock agnostic' technologies such as pyrolysis, gasification and hydrothermal liquefaction so that multiple feedstocks can be used in integrated biofuel production facilities and carbon from both lignin and wall polysaccharides can be simultaneously converted to hydrocarbons. The fact remains that cell wall polysaccharides and lignin represent the most abundant renewable sources of carbon on the planet, and it is equally certain that we will continue to pursue biofuel production from these sources and that we are developing an acute understanding of the fundamental science of cell wall biology that will enable us to engineer new plant feedstocks to fit the needs of the production system. One can predict too that cell wall polysaccharides will continue to be important in human diets, as more information on the health benefits and mechanisms of action of dietary fibre is obtained. Metagenomics technologies will help us understand the consequences that wall components in the diet bring to the compositions of microbiota populations and metabolic processes that occur in the large intestine, and how these in turn affect bowel health. Emerging information on the role of short chain fatty acids in reducing the risk of inflammatory diseases such as emphysema and asthma will provide further impetus to increasing the dietary fibre of human diets. It might also be possible to engineer beneficial large intestine microorganisms to better compete for incoming carbon from a range of wall polysaccharides. Drawing kindly provided by Bruno Carrocci.

levels of primary and secondary cell wall cellulose synthesis [8]. These suggestions have been based largely on indirect evidence from co-expression analyses of *CesA* genes and from antibody co-precipitation. Zinc-finger domains on cellulose synthases are consistent with the formation of a multi-protein complex that could not only involve the cellulose synthase enzymes themselves, but might include other proteins. In this context, proteins such as KORRIGAN and COBRA have been implicated in cellulose biosynthesis and other proteins required for transport through the endomembrane system and for interactions with cortical microtubules have been identified.

In considering cellulose synthase complexes it is important to address the issue of cellulose crystallinity, because perturbation of cellulose synthesis *in planta* in many cases causes changes in measured crystallinity. Indeed, disorder of cellulose in chain packing and at the microfibril surface contribute to the departure from 'crystalline' cellulose, although the degree of crystallinity depends on the method used to measure it, be that spectroscopic, diffraction or chemical extraction [7••]. Differences in crystallinity could result from a number of biological processes, including the disposition and stability of the cellulose synthase complex, the presence of interacting polysaccharides in the wall and the possible involvement of hydrolytic enzymes in trimming away non-crystalline regions. [9].

Molecular mechanisms of cellulose synthesis

A recent highlight in cell wall biology was the publication of the three-dimensional structure of a membrane-bound cellulose synthase complex from the bacterium *Rhodobacter sphaeroides*, in which a 1:1 complex of a catalytic BcsA subunit and a membrane-anchored BcsB protein form an active enzyme complex containing a nascent cellulosic chain [10••]. The BcsA subunit binds the uridine diphosphate-glucose substrate on the