Genetic Modification of Plant Cell Walls for Enhanced Biomass Production and Utilization

Investigators

Chris Somerville, Professor, Department of Biological Sciences, and Director, Carnegie Institution, Department of Plant Biology; Jennifer Milne, Postdoctoral Fellow, Department of Biological Sciences

Introduction

In 2002, The US Department of Energy released a document entitled "The Vision for Bioenergy and Biobased Products in the US" that envisioned 5% of energy, 20% of transportation fuel and 25% of chemicals being derived from biomass by 2030. A crucial factor in reaching these goals will be the net productivity of the plants used. It is generally accepted that the current practice of converting excess starch production to ethanol does not represent a significant long-term opportunity. By contrast harvesting the "cellulosic biomass" that comprises the body of plants represents an attractive target. A recent study by DOE and USDA estimated that there is approximately 1.4 billion dry tons per year of renewable excess biomass capacity in the US. This is roughly equivalent to the energy content of all imported petroleum.

Plant biomass is primarily a mixture of six types of polysaccharides. These can be utilized for energy production in a variety of ways, including direct combustion, gasification, pyrolysis, and enzymatic conversion to ethanol or other organics. In order to maximize the energy efficiency of biomass production it is essential to minimize requirements for fixed nitrogen (~30 Gj/tonne), which is required primarily for protein and lignin synthesis. The polysaccharides that comprise the bulk of biomass do not contain any nitrogen. Thus, from a system engineering perspective, it is desirable to maximize polysaccharide accumulation while minimizing demand for nitrogen. Because cellulose is main component of biomass, an increased carbon/nitrogen ratio may be accomplished by increasing the amount of cellulose per cell relative to other components. Since light capture and photosynthetic electron transport are not limiting, there is excess carbon flux available to support increased cellulose synthesis. By modifying the normal biological control of cellulose synthesis, we think it possible todevelop plants that accumulate more fixed carbon per unit land area per unit time with no increased nutritional requirements.

The proposed experiments are focused on the model plant Arabidopsis thaliana. This plant has no direct utility. However, it is a typical higher plant with many technical advantages for experimental work that include a fully sequenced genome, full-genome DNA chips fro measuring gene expression, a collection of sequence-indexed insertion mutations, a large collection of characterized mutations, a large literature and a large research community. Knowledge gained from basic research with Arabidopsis can be readily translated to other plants.

This project will begin in June 2005.

Background

The leaf cell walls of a dicot species such as Arabidopsis contain three major classes of polysaccharides: cellulose, hemicelluloses, and pectins. Cellulose is present as long unbranched fibrils composed of approximately 30 to 36 hydrogen-bonded chains of β-1,4-glucose. Hemicelluloses are branched polysaccharides containing backbones of neutral sugars that can form hydrogen bonds to cellulose fibrils. Pectins are complex polymers that contain uronic acids as major components. The amounts of the various polymers vary from one cell type to another for largely unknown reasons. Notably, the amount of cellulose may vary many-fold from one cell type to another. This is most readily evident in vascular tissue where the xylem cells exhibit "secondary cell walls" that are evident as a second layer of deposition within the "primary wall".

A representative structure for an Arabidopsis leaf primary cell wall is presented in Figure 1. The complexity of the image underscores the challenge associated with understanding the structure, function and synthesis of plant cell walls. The cellulose microfibrils are insoluble and are made at the plasma membrane. The other polymers are secreted and diffuse within the aqueous environment of the wall to their final destination. A driving force for wall assembly is thought to be the hydrogen bonding of hemicellulose to cellulose microfibrils.
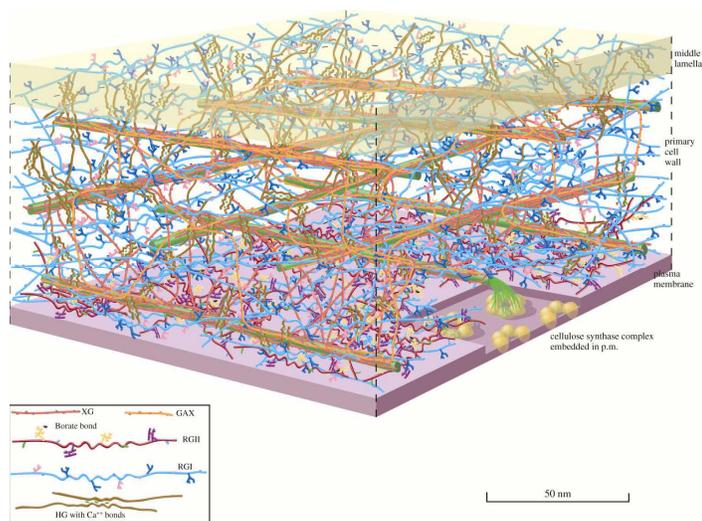

Figure 1: Scale model of the polysaccharides in an Arabidopsis leaf cell. The amount of the various polymers is shown based approximately on their ratio to the amount of cellulose. The amount of cellulose shown was reduced, relative to a living cell for clarity. From Somerville et al., (2004).

The model species, Arabidopsis, has a type I wall typical of most higher plants making it a good genetic model for understanding cell wall synthesis, structure and function.   In leaf mesophyll cells of Arabidopsis, cellulose comprises only about 14% of total wall mass. By contrast, in xylem cells, cellulose appears to form the majority of the mass. Thus, the amount of cellulose per cell is under developmental control and it is apparent that increased amounts of cellulose are not necessarily incompatible with cellular function.

Cellulose synthase is located in the plasma membrane. A family of genes encoding the catalytic subunit of higher plant cellulose synthase, termed *CESA*1-10 have been identified. In addition, mutant analysis has identified a number of other genes that contribute indirectly to the overall process. Analysis of where and when the various *CESA* genes are expressed combined with genetic analyses indicate that three CESA genes (CESA4, 7, 8) carry out the synthesis of cellulose in cells that undergo secondary cell wall thickening. We will test the hypothesis that cellulose synthesis is controlled at the level of gene expression by producing and characterizing transgenic plants in which the genes that control secondary cell wall synthesis are expressed in cells that do not normally produce secondary cell walls.

Future Plans
The goal is to express the three CESA genes that make secondary cell wall cellulose in cells that do not normally have secondary wall thickening. Because it is likely to be deleterious to induce extra cellulose synthesis in cells that need to divide and expand to support normal growth and development, the genes must be placed under transcriptional control of a promoter that is active at a time that is compatible with normal development. We will use a chemically-inducible promoter (i.e., dexamethasone inducible) so that we can induce transcription by exogenous application of an inducing chemical. This will allow production and propagation of the transgenic plants and will also facilitate studies of the consequences of inducing expression of the *CESA* genes at specific time and places and to differencing degrees. Additionally, we will use a developmentally controlled promoter that is inactive in expanding cells or cells that are fated to divide and expand at a later stage of development. Transgenic plants will be produced by standard methods. We will screen the resulting population of transgenic plants for individuals in which each of the three genes is expressed under appropriate conditions. In order to test whether each of the CESA proteins is expressed, we will modify the corresponding genes so that the proteins carry a distinct epitope (i.e., MYC, FLAG, or HIS) near the N-terminus.

The transgenic plants containing the ectopic *CESA* genes will be analyzed for cell wall composition (eg., cellulose and other polymers) and for effects on growth and development. If quantitative differences are observed, we will visualize the walls by transmission electron microscopy to determine whether the additional cellulose is morphologically distinguishable. Many additional questions would then emerge such as the molecular weight of the cellulose, the orientation of the fibrils, the presence in the increased layer of other polysaccharides (as visualized by immuno electron microscopy using antibodies against various polymers). Additionally, in order to examine whether the

transgenic plants sense and respond to the abnormal expression of the *CESA* genes we will examine effects on gene expression using whole genome DNA chips.

If increased cellulose is obtained from chemical induction of the genes, we will test the feasibility of engineering enhanced cellulose under the control of developmental stage-specific promoters. Based on an extensive analysis of tissue specific gene expression which we carried out for other purposes, we have identified a number of candidate promoters as noted above (ie., high expression in nondividing and non expanding cells and no expression in expanding cells). The design and production of the *CESA* constructs will be identical to that using the Dexamethasone-inducible promoter. Transgenics with high, medium and low levels of expression will be identified by screening by real-time PCR. The analysis of the transgenics will be similar to that using the Dexamethasone-inducible genes except that we will be confined to testing various tissues of the plants throughout the life cycle.

References

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Contacts
Chris Somerville: crs@stanford.edu
Jennifer Milne: jmilne@stanford.edu