

ANALYSIS

Building better trees with antisense

Ron Sederoff

Forest trees are one of the world's most important natural resources. As crop plants, they are in the earliest stages of domestication, and much of our wood is still harvested from natural forests. Until now, the types of genetic changes that made possible the domestication of agricultural crops have not been possible for trees because hundreds or thousands of generations are required for selection. Biotechnology provides the opportunity to make such changes through genetic engineering in a matter of years, rather than centuries.

In this issue, Vincent Chiang and colleagues¹ show that an antisense construct for a gene in the lignin pathway can greatly reduce lignin, increase cellulose, and dramatically stimulate growth in transgenic aspen. Such changes could be important in tree domestication and could ultimately lead to trees that are profoundly different from their current undomesticated progenitors.

Fast-growing, low-lignin trees could provide significant practical benefits. Removal of lignin from the wood cell walls is the most energy intensive and environmentally damaging step in wood processing for pulp and paper, so reducing the lignin content in trees could provide both economic and environmental benefits. The money derived from wood and wood products represents more than 1% of the world's economy², with roughly equal amounts of wood being used for industrial products, such as pulp and paper, and for fuel.

Although aspen is not one of the most commercially valued trees, if the results achieved by Chiang and colleagues are extended to more widely planted trees, such as eucalyptus or pines, they will have a

very large economic impact globally. Even more importantly, any increase in efficiency that allows production of more wood and wood products from less land helps conserve natural forests and reduces the environmental impact of processing wood into pulp and paper³.

Chiang and colleagues were able to

tent. Some workers have argued that lignin polymerization is highly regulated⁴; however, the great variation in lignin composition suggests considerable metabolic plasticity⁵.

In their previous research, Chiang and colleagues⁶ isolated and characterized two enzymes from an earlier step in the lignin biosynthetic pathway of quaking aspen (*Populus tremuloides*). Both are isozymes of 4-coumarate:coenzyme A ligase (4CL), which activates cinnamic acids by ligation of coenzyme A (Fig. 1). The 4CL enzyme is also the phenylpropanoid precursor for flavonoid biosynthesis and constitutes a branch point between flavonoids and lignin. The gene for one of these isozymes, *Pt4CL 2*, is expressed in the epidermis of stems and leaves, presumably for biosynthesis of flavonoids, whereas the other, *Pt4CL 1*, is expressed in differentiating xylem, presumably for lignin biosynthesis.

In the present study, Chiang's group expressed an antisense construct to downregulate the gene for the xylem form of 4CL (*Pt4CL 1*), and demonstrated a profound effect on wood composition and tree growth: At 10 months of age, the transgenic aspens contained up to 45% less lignin and as much as 15% more cellulose than nontransgenic aspens; and the growth of the transgenic aspens was substantially enhanced. The composition of lignin and the cellular morphology were unchanged.

These results raise interesting issues concerning the regulation of the lignin biosynthetic pathway and the formation of the secondary cell wall. The observation that an inhibition of 4CL activity both reduces lignin content and increases content of the nonlignin cell wall phenolics suggests that this enzyme catalyzes a pivotal step for most, if not all, of the different precursors for lignin. Moreover, the transgenic trees show other changes in wood composition that reveal a link between the different biosynthetic pathways for the major compo-

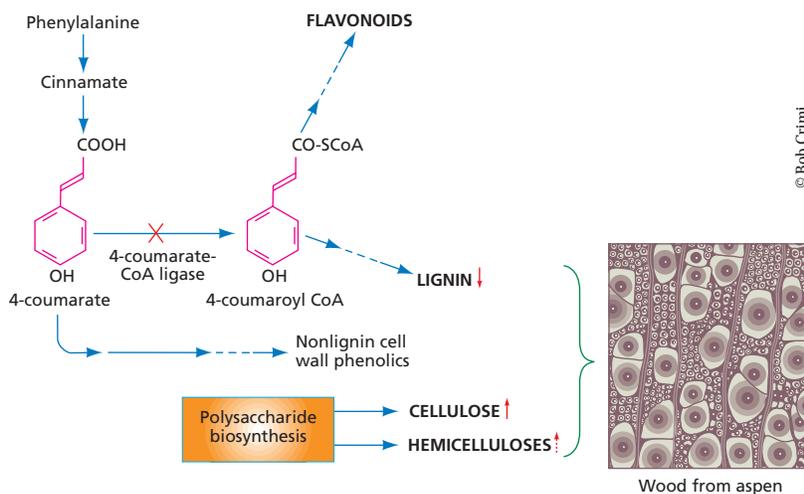


Figure 1. Pathway for lignin biosynthesis. The 4-coumarate:coenzyme A (CoA) ligase (4CL) leads to a major branch point in phenylpropanoid metabolism. The product 4-coumaroyl:CoA is a precursor for lignin and flavonoids. Other cinnamic acids such as ferulic acid are similarly activated (see Fig. 1 of Hu et al.¹, this issue). Lignin, cellulose, and hemicellulose form the cell walls of xylem, which transports water and supports the tree. When expression of the gene encoding 4CL is downregulated in trembling aspen (*Populus tremuloides*), lignin content is decreased, cellulose is increased, and growth is stimulated.

manipulate tree growth and wood formation by modifying the biosynthesis of a specialized multilayered cell wall. The wood cell wall is a reinforced composite with cellulose as the fibrous strength-bearing element embedded in a network of smaller carbohydrates (hemicelluloses), and lignin, a highly hydrophobic, crosslinked phenolic polymer. The pathway for lignin biosynthesis (Fig. 1 of Hu et al.) is one of the most intensively studied in higher plants. The precursors of the polymerization of lignin (monolignols) are derived from cinnamic acid, which is formed from the deamination of phenylalanine. Cinnamic acid is modified by hydroxylation of the ring, subsequent methoxylation, and reduction of the modified cinnamic acids to cinnamyl alcohols, the monolignol precursors for lignin.

Previous attempts to modify lignin by mutation or suppression of enzymes in the pathway for monolignol biosynthesis have resulted in striking changes in lignin composition, but usually with no effect on lignin con-

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Wood from aspen

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nents of the wood cell wall. Lignin is reduced, cellulose increased, and selective increases occur in the hemicellulose components arabinan, galactan, and rhamnan, whereas the xylan component remains unaffected. The interrelatedness of these responses indicate important molecular interactions that have yet to be defined.

Most intriguing is the enhanced growth of the transgenic trees. Previous studies suggested that even large changes in lignin composition can occur without impairing growth^{5,7}. In the present study, the lignin content, not composition, was altered. The fact that the extent of growth enhancement was not directly correlated with lignin content prompted the authors to propose that other pathways other than lignin biosynthe-

sis may be involved. The absence of an explanation for the effect of reduced lignin on growth does not detract from its potential impact as a possible way for controlling growth and development in trees. These results further support the view that the potential for modifying wood properties through genetic engineering may be far greater than first anticipated⁷.

The next step will be to follow these trees through to maturation and see how they perform in the field through successive generations, particularly for wood properties and resistance to pests and pathogens. The results of the research by Chiang and his colleagues will stimulate the creation of many new transgenic tree varieties using genes important in phenolic

metabolism and cell wall biosynthesis. We should look for variants of these genes in non-transgenic trees as well. It will be exciting to see the new varieties tested. On the way, we might be able to start seeing the forest, the wood, and the trees.

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Hybridization prediction gets to first base

C.A. Stein

All oligonucleotide-based technologies that target mRNAs, from microarray analysis to antisense- or ribozyme-based strategies for gene therapy, require Watson-Crick base pair binding of the effector molecules to their target sequence. However, there are virtually an infinite number of potential target sequences contained within any given mRNA. Unfortunately, despite some elegant computational attempts¹, there currently is no way of readily identifying optimum target sites, or even those that are simply active. If antisense technologies are ever to make the big leagues, the factors that determine the characteristics of heteroduplex formation must be established. In this issue, Mir and Southern² have successfully used a rationally designed oligonucleotide array to identify oligodeoxynucleotides than optimally hybridize to a target tRNA. While not a home run, their approach is a good solid single and perhaps the start of a rally.

The crux of the problem facing the genomics community is that with the anticipated completion of the Human Genome Project, tens of thousands of gene sequences will be available, and RNA-directed oligonucleotide technologies, at least in theory, offer some of the best methods for the determination of gene function. But the frustrating bottleneck of determining an optimal targeting region on the mRNA has severely limited these kinds of exciting applications, and will

continue to do so unless wasteful empiric targeting strategies can be eliminated.

In antisense oligonucleotide experiments¹, the choice of target is purely empirical; no way currently exists to know *a priori* which sites on the mRNA molecule should be targeted. Experimental success can only be achieved by a “brute force” approach: once the sequence of the target mRNA is determined, approximately 20–40 antisense oligonucleotides, derived solely by mRNA walking (i.e., substituting a sequence with an adjacent one in a stepwise manner), need to be synthesized in order to obtain two to four active antisense molecules^{3,4}. Invariably, the majority of the oligonucleotides are ineffective. The synthesis, purification, and evaluation of several dozen candidate antisense (or ribozyme or external guide sequence; EGS) effectors is laborious and expensive, and therefore ill-suited to high-throughput development.

Mir and Southern now step up to the plate with an inspired approach to this



Figure 1. Schematic representation of the binding of tRNA^{Phe} to an array of complimentary oligonucleotides immobilized on a glass plate. A radiolabel attached to the tRNA allowed an assessment of binding to individual oligonucleotides within the array.

tricky problem. Although they are not the first to do so⁵, they have postulated that the structure and not simply the sequence of the target RNA, determines which sites can form stable heteroduplexes. To test this idea, they employed an immobilized oligonucleotide array synthesized on a glass plate, a technology which was developed in Southern's laboratory⁶. The target RNA that was used to probe the array was radiolabeled tRNA^{Phe}, which has been extensively studied and for which a crystal structure has been solved. It was therefore a relatively straightforward matter for the authors to determine which of the myriad of oligonucleotides immobilized to the glass plate bound with high affinity to the target.

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