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Lignin bioengineering☆

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Lignin is one of the most abundant aromatic biopolymers and a major component of plant cell walls. It occurs via oxidative coupling of monolignols, which are synthesized from the phenylpropanoid pathway. Lignin is the primary material responsible for biomass recalcitrance, has almost no industrial utility, and cannot be simply removed from growing plants without causing serious developmental defects. Fortunately, recent studies report that lignin composition and distribution can be manipulated to a certain extent by using tissue-specific promoters to reduce its recalcitrance, change its biophysical properties, and increase its commercial value. Moreover, the emergence of novel synthetic biology tools to achieve biological control using genome bioediting technologies and tight regulation of transgene expression opens new doors for engineering. This review focuses on lignin bioengineering strategies and describes emerging technologies that could be used to generate tomorrow's bioenergy and biochemical crops.

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Introduction

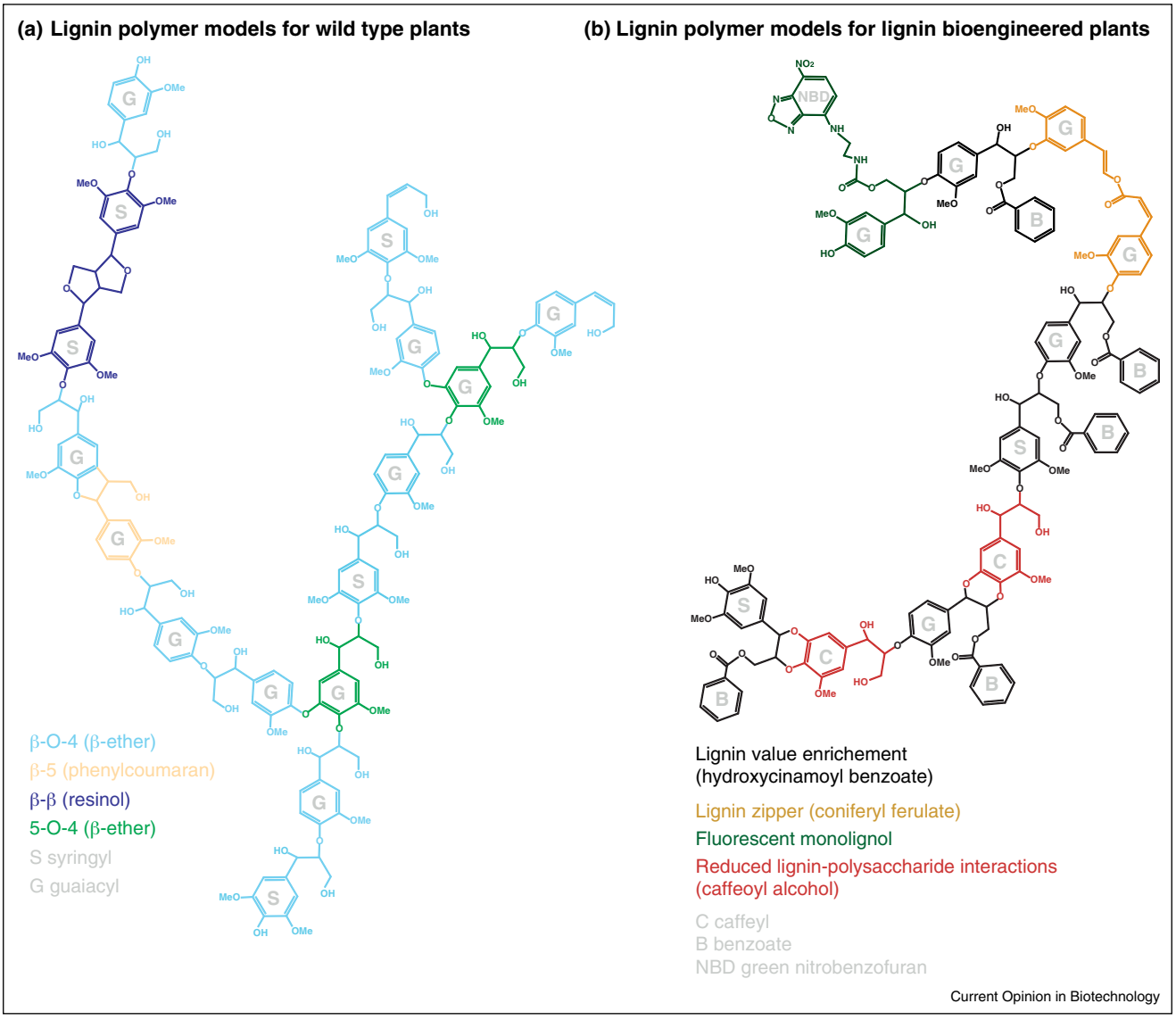
In its effort to make cellulosic biofuel production more cost-effective, the bioenergy field has necessarily focused much of its attention on plant cell walls. Lignin, a major component of cell walls, is the third most-abundant biopolymer and the largest available resource of natural aromatic polymers (Figure 1a). It is mainly composed of the monolignols *p*-coumaryl, coniferyl, and sinapyl alcohols which give rise to the *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignin units [1]. Unfortunately, it is also the primary contributor to the high cost of lignocellulosic sugar production, because cell wall polysaccharides are encrusted with lignin which make them highly resistant to extraction and enzymatic hydrolysis [1,2]. Moreover, lignin has almost no commercial value aside from its role as a source of heat, and it is generally treated as a waste product [3].

Lignin has been a target of genetic manipulation for several decades because its content in biomass is inversely correlated with its forage digestibility and kappa value in the pulping industry [4,5]. Lignin biosynthesis is well-characterized and all the enzymes required for the synthesis of its three major building blocks — called monolignols — are well-known and highly-conserved in all vascular plants [6,7]. Unfortunately, lignin cannot be simply removed from growing plants without causing deleterious developmental effects [8]. Genetic manipulation trials using natural mutants or silencing strategies have failed because they drastically reduced lignin content in a non-selective way. Nevertheless, there are cases in which mild genetic manipulations have been used to moderately reduce lignin content or modify its composition in biomass, modestly improving saccharification efficiency, forage digestibility, and pulping yield [9]. These approaches are still rather limited.

Novel strategies need to be developed to reduce lignin content further, without altering plant development or causing undesirable effects. Classical lignin-modification methods typically repress the expression or activity of lignin biosynthetic genes. They require identification of natural defective alleles, the screening of single-nucleotide polymorphisms (SNPs) from mutant populations (usually a labor-intensive process) or the development of RNAi-based gene-silencing approaches. The limit of all these approaches is the lack of tissue specificity because every cell carries the same defective allele or silenced gene since RNAi move from cell-to-cell and affect most of the tissues in the plant [10]. Moreover, they affect not only the lignin biosynthesis pathway, but also have indirect effects on other metabolic routes connected to the phenylpropanoid and monolignol pathways. The phenylpropanoid pathway, for example, generates a wide array of secondary metabolites that contribute to all aspects of plant development and plant responses to biotic and abiotic stresses [11].

Recently, researchers have developed more elaborate approaches for lignin modification and employed tissue-specific promoters to reduce the risk of disturbing other phenylpropanoid-derived pathways in non-lignified tissues [12••,13••]. The utilization of such promoters is

Figure 1



Lignin polymer models. **(a)** Lignin polymer models for wild type plants; **(b)** lignin polymer models for lignin bioengineered plants. Bioengineered lignin is exclusively composed of representative unusual monolignols to increase lignin value; to facilitate lignin degradation (lignin zipper); to reduce lignin–polysaccharide interactions; or to fluorescently label lignin.

challenging because most of the lignin genes (PAL, C4H, 4CL, HCT, C3H, among others) belong to the phenylpropanoid pathway [14•]. Use of the corresponding promoters for engineering purposes may affect the biosynthesis of associated metabolites such as flavonoids, suberin, coumarins, phenolic volatiles, or hydrolyzable tannins. On the other hand, most promoters of secondary cell-wall biosynthetic genes (CesAs, GTs, or lignin genes) [15] are expressed in both vascular bundles and interfascicular xylem fibers, raising concerns that lignin modification would affect the integrity of vessels. Vessel-specific and fiber-specific genes (and corresponding promoters) were identified in few species and their number remains limited (VNDs, NSTs, SNDs, WNDs, Lac17 [16–20]). Single-promoter-driven transgene expression, which can confer both adequate spatio-temporal expression and transcription strength for optimal engineering, is consequently difficult to achieve. Furthermore, using several copies of the same promoters for engineering may lead to silencing issues, including the silencing of endogenous promoters if they share high sequence similarities. However, adjusting transgene expression to optimal levels and restricting it to specific cells at particular developmental stages will reduce undesirable side effects. Ideally, newly emerging techniques will be combined with tissue-specific promoters to meet the challenges associated with plant