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Review

# Lignification: Flexibility, Biosynthesis and Regulation

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**Lignin is a complex phenolic polymer that is deposited in the secondary cell wall of all vascular plants. The evolution of lignin is considered to be a critical event during vascular plant development, because lignin provides mechanical strength, rigidity, and hydrophobicity to secondary cell walls to allow plants to grow tall and transport water and nutrients over a long distance. In recent years, great research efforts have been made to genetically alter lignin biosynthesis to improve biomass degradability for the production of second-generation biofuels. This global focus on lignin research has significantly advanced our understanding of the lignification process. Based on these advances, here I provide an overview of lignin composition, the biosynthesis pathway and its regulation.**

## Lignification Flexibility

Lignification is a complex process found only in higher plants, with the main functions to provide stability to the vascular part of the plant and form a barrier against microbial infections. Lignin monomer biosynthesis starts with the deamination of the aromatic amino acid phenylalanine, followed by a series of hydroxylation, methylation and reduction resulting in the production of the three most common basic units of the lignin complex. Natural lignin polymer generally comprises *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, which are produced by three primary monolignols, *p*-coumaryl, coniferyl and sinapyl alcohols respectively (Figure 1). However, lignification shows considerable flexibility to incorporate 'nontraditional' (other than H, G, and S units) monomer units. For instance, the hydroxycinnamaldyhes, hydroxycinnamic acids, dihydroconiferyl alcohol, and ferulate monolignols were all identified in lignin polymers that exist in small amounts in various plant species [1–3]. Lignin polymers are highly resistant to enzymatic degradation and therefore, the recalcitrance of lignin polymers acts as a major obstacle to improving forage crop nutritional quality and industrial processing. With substantial research efforts aiming to manipulate the lignin biosynthesis pathway in bioenergy crops, more and more 'artificially designed compounds' have been successfully introduced into the plant secondary cell wall as alternative lignin monomers. Those examples were nicely summarized in a recent review about lignin engineering [4]. Here, I cover the overturning of some established views of lignin biosynthesis as well as a new appreciation of the plasticity of lignin composition.

## Lignin Polymerization

Lignin polymerization has inherent plasticity, but the traditional monomers have priority in the lignification process. However, the variation in lignin composition can be extensive because lignin can comprise nearly any homopolymer rather than just the three natural monomers. Downregulation of ferulate 5-hydroxylase (*F5H*) or caffeic acid *O*-methyltransferase (*COMT*) eliminates S subunits and overexpression of F5H in a tissue-specific manner results in nearly all S subunits [5,6]. Simultaneous mutation of the Mediator complex subunits MED5a and MED5b rescues the stunted growth of the arabidopsis (*Arabidopsis thaliana*) mutant *p*-coumaroylshikimate 3′-hydroxylase (C3'H) and the triple mutant comprises almost exclusively H units,

### Trends

The eukaryotic Mediator complex is a conserved central component of the transcriptional machinery.

Recently, plant Mediator subunits have been reported to participate in numerous biological processes, such as plant defense, noncoding RNA production, and cold tolerance.

Originally reported as REDUCED EPIDERMAL FLURESCENCE 4 (*REF4*) and REF4-RELATED 1 (*RFR1*), now renamed as *MED5a* and *MED5b*, these two genes are suggested to be critical for phenylpropanoid homeostasis.

It is possible that MED5a and MED5b regulate lignin biosynthetic genes translationally or repress the gene transcription in the step of lignin monomer transport or polymerization.

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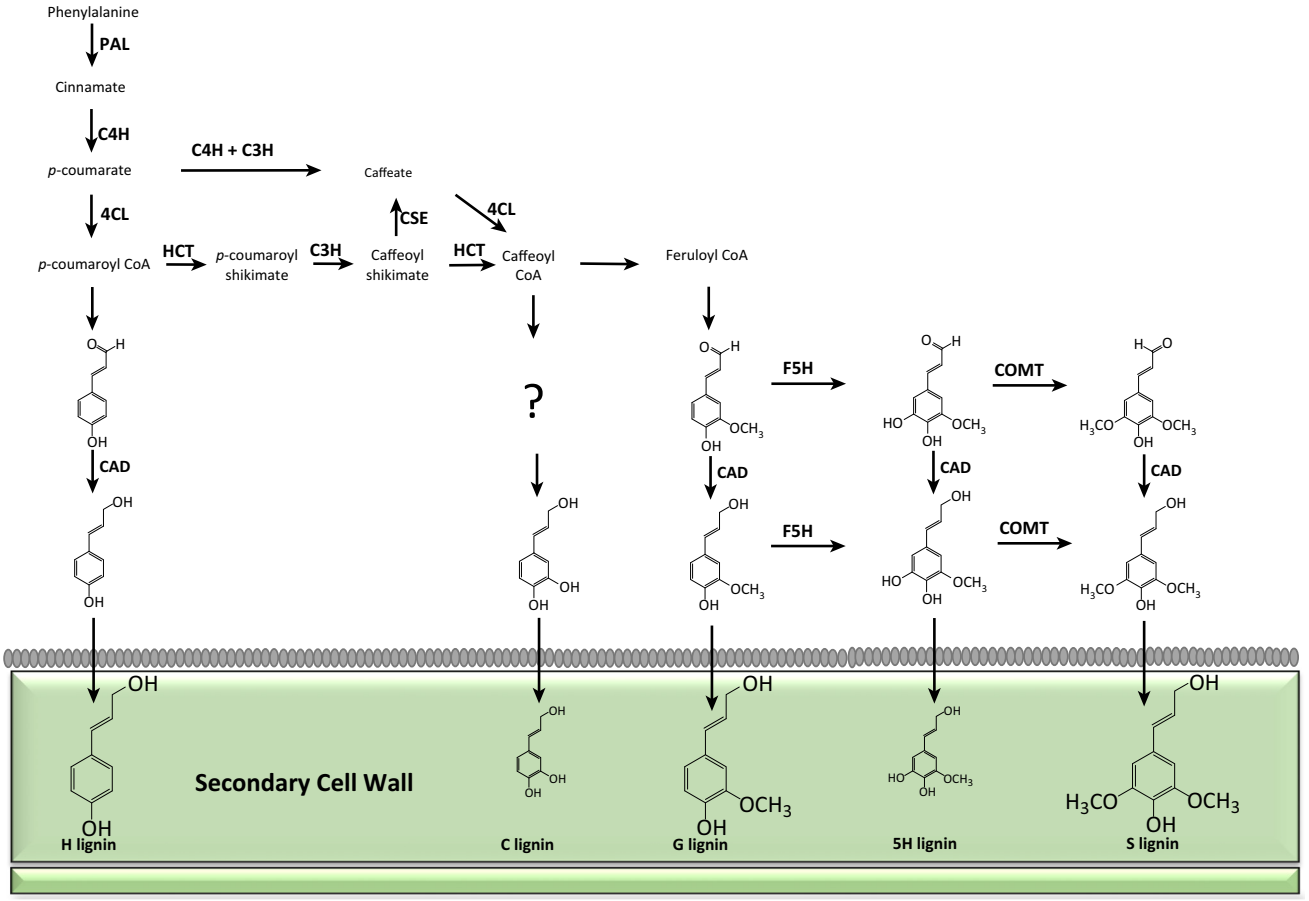


Figure 1. General Steps in Lignification via the Phenylpropanoid Pathway. The names of the enzymes and intermediates are listed based on current knowledge of the biosynthetic pathway. The enzymes above each arrow are responsible for catalyzing the corresponding steps. H, G and S lignin monomers are the basic units of lignin polymers. However, the presence of C and 5H lignins has also been reported in some plant species. The relatively small sizes of the chemical structures of the C and 5H lignins compared with the H, G and S lignin monomers indicate that C and 5H lignin monomers are uncommon. Abbreviations: 4CL, 4-coumarate:CoA ligase; C3H, *p*-coumaroyl shikimate 3′-hydroxylase; C4H, cinnamate 4-hydroxylase; CAD, cinnamyl alcohol dehydrogenase; COMT, caffeic acid/5-hydroxyconiferaldehyde 3-*O*-methyltransferase; CSE, caffeoyl shikimate esterase; F5H, ferulate/coniferaldehyde 5-hydroxylase; HCT, hydroxycinnamoyl CoA:shikimate hydroxycinnamoyl transferase; PAL, L-phenylalanine ammonia-lyase.

whereas these units account for only around 2% of natural lignin in angiosperms [7]. To the best of my knowledge, this is the first evidence showing that plants are able to grow normally with only the *p*-hydroxyphenyl-type lignin. The catechyl (C) unit is also found naturally in seed coats of the vanilla orchid as a homopolymer and in some members of the Cactaceae [8,9]. Interestingly, the C unit appears to be independently regulated compared with traditional G and S units [9]. The *Medicago truncatula* cinnamyl alcohol dehydrogenase (CAD) mutant surprisingly contains more than 95% hydroxycinnamaldehyde-derived lignin units, whereas natural lignin has only around 5% hydroxycinnamaldehyde and only at the ends of the polymers [10].

Even though lignin polymerization can occur with nontraditional units, G and S units are always preferred when available. In an attempt to design novel lignins for improved biofuel production, monolignol ferulates were successfully introduced into the cell wall of poplar trees, but the percentage of the ferulate conjugate was less than 25% [3]. Overexpression of *F5H* in a *COMT* loss-of-function mutant led to the accumulation of 5-hydroxy-G units (5H). However, compared with nearly 90% S lignin in overexpression of *F5H* alone, overexpressing *F5H* in the *COMT*-deficient mutant resulted in <30% of 5H units and most (around 70%) were still G units