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LIGNINS: BIOSYNTHESIS, BIODEGRADATION AND BIOENGINEERING

Volume 61

Edited by
LISE JOUANIN
and CATHERINE LAPIERRE

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Volume Editors

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DEDICATION

As this book neared completion, we learnt of the sudden death of Prof. Alfonso Ros Barcelo. Just before his demise, Prof. Barcelo sent us the comprehensive chapter about the evolutionary history of lignins. As coeditors, we deem it a privilege to have received this outstanding contribution from Prof. Barcelo's group and we dedicate this book to the memory of Prof. Barcelo whose research and scientific insight greatly contributed to recent advances in the understanding of lignin biosynthesis.

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PREFACE

Lignification, that is, the deposition of lignins in cell walls, is linked to the colonization of land by plants. This major event of the plant kingdom had such unique outcomes for mankind that we cannot imagine a lignin-less world. Lignified plants probably allowed early humans to get the control of fire. Man-made products from lignified plants had other outstanding roles, such as the worldwide dissemination of human knowledge on paper invented in China some 2000 years ago. As regards future, lignified plants represent huge, renewable and sustainable feedstocks for energy, chemicals and materials. This was anticipated in 1876 by Fremy in his lecture to the French Academy of Sciences, when he declared "When the composition of the skeleton of plants is known, it will be easy to help the various industries challenged with the production of alcohol or pulp from wood or straw". The current development of biorefineries from plant cell walls (i.e. plant "skeleton") remarkably echoes this vision.

As quoted in most papers about lignins, these unique Nature's aromatic polymers are the second most abundant organic constituents of the biosphere, next to cellulose. Lignification mainly occurs in the walls of terrestrial vascular plants, mainly in the secondarily thickened cells of supportive or conductive tissues which thus acquire novel properties, that is, rigidity, impermeability and decay resistance. By providing mechanical support to plant stems, enabling water conduction from roots to leaves, and protecting plants against pathogens, lignins are essential to living woody or herbaceous vascular plants, but they may have opposite effects on their postharvest uses. Lignins positively impact the properties of wood when used as firewood or timber. However, they detrimentally affect the chemical production of pulp, the nutritional value of forages or the biological production of cellulose-derived fuel ethanol.

Lignins have been studied for more than 150 years and the extensive output of this research has been collected in some landmark textbooks such as the multiauthored *Lignins*, edited by Sarkanen and Ludwig in 1971. Before the 1990s, most studies focused on lignin chemistry and biochemistry and their applied issues. Since two decades, the molecular biology and genetic engineering applied to the field of lignification have become a major support for lignin research.

The explosive growth of molecular biology has markedly reassessed our view of the biochemical pathway leading to lignins (Fig. 1). Many lignin features can be changed by genetic alterations of this pathway: concentration, distribution, structure, interacting capabilities, susceptibility to various

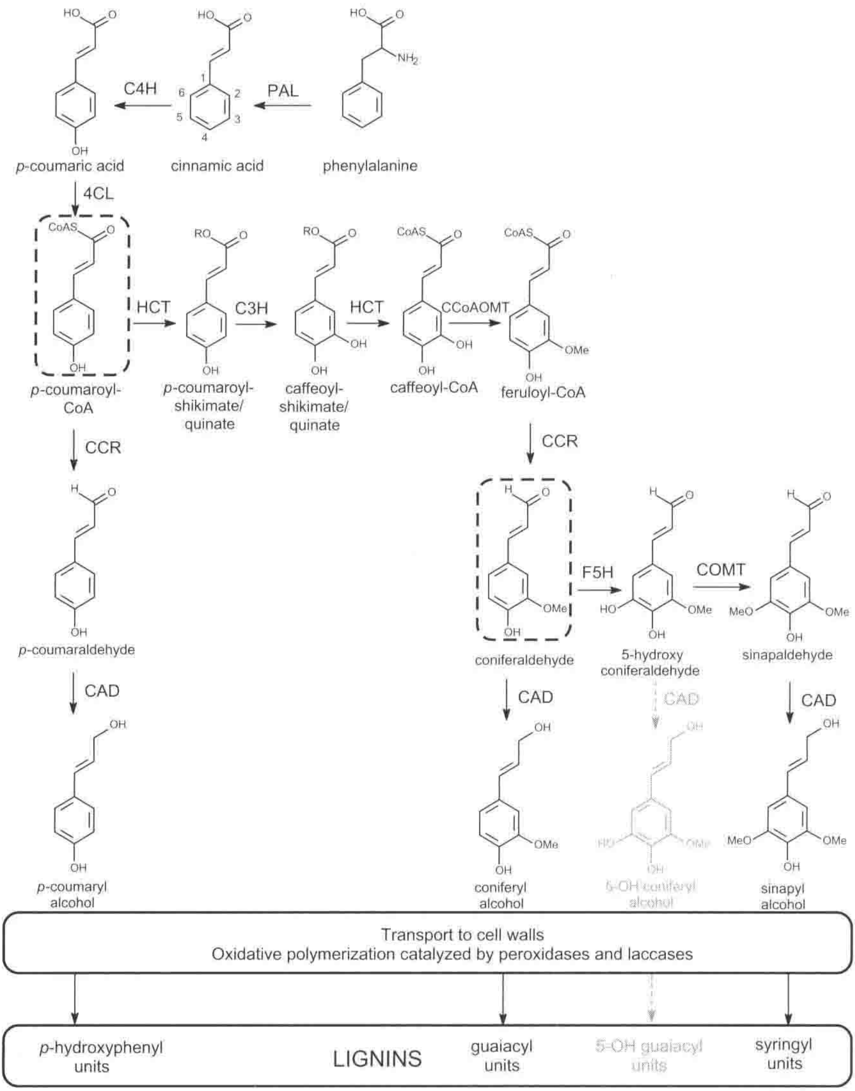

Fig. 1. Current and simplified view of the biosynthetic pathway going from phenylalanine (Phe) to lignins. This pathway includes many steps of reduction, (thio)esterification, hydroxylation and methylation, to yield *p*-coumaryl, coniferyl and sinapyl alcohols, the respective precursors (or monolignols) of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignin units. The significance of every step of this pathway has been studied by means of appropriate mutant or transgenic plant. In angiosperms silenced for caffeic acid *O*-methyltransferase (COMT), there is a shortage in sinapyl alcohol, the precursor of S units, and an accumulation of 5-OH coniferyl alcohol (in grey) then incorporated into lignins as 5-OH guaiacyl units (in grey). Silencing the various actors of the lignin pathway may have several effects: decreased lignin content; modification of the relative frequency of H, G or S

treatments, etc. A milestone to the understanding of how lignins can be genetically designed was achieved in 1988, with the identification of unusual lignins in a maize mutant altered in *O*-methyltransferase activity. In this mutant and beside the conventional lignin units, we discovered unusual 5-OH guaiacyl units (Fig. 1). This discovery was the first support to lignin chemical plasticity, a concept which refers to the ability of plants to produce operational lignins by the incorporation of unusual precursors when there is a genetically induced deficit of conventional ones. This result opened the way to select and design lignified cell walls more adapted to human needs. Today and in model species (mainly tobacco, Arabidopsis, poplar and, more recently, *Brachypodium distachyon*), plants deregulated for most, if not all, known lignin-related genes have been produced and subjected to the extensive evaluation of the consequences of their altered lignification.

This book is by no means a comprehensive treatise covering the entire field of lignins, but rather gives a special emphasis to the bioengineering of these enigmatic polymers. It is divided in nine chapters containing up-to-date reviews by expert groups in their field.

Lignin-modified transgenic trees are crucial for evaluating the consequences of altered lignification on their fitness, environmental effects, and industrial performances. This hotly debated issue is addressed in the first two chapters. In the first chapter, Pilate *et al.* make a comprehensive review of field trials with lignin-modified transgenic trees and provide their personal vision about past studies and some clues for tree biotechnology. In the second chapter, Wagner *et al.* more specifically focus on lignin manipulation in conifers and point out some specific strategies for lignin design in these trees of great ecological or economic value.

Nearly one-third of the land vegetative cover is dominated by grass ecosystems. The unique specificity of grass cell walls, relative to nongrass walls, is the participation of *p*-coumaric and ferulic acids to the wall assembly. As the biosynthetic pathways to these acids and to lignins share some similarities, this peculiarity makes the genetic designing of grass lignins still more challenging. The molecular biology of lignification in grasses is presented in

conventional units; incorporation of unusual units and/or redirection of carbon flux into nonlignin phenolics. Two intermediates are recognized as being at metabolic crossroads: (i) *p*-coumaroyl CoA, which is dedicated to flavonoids by chalcone synthase (not shown), H units by cinnamoyl-CoA reductase (CCR) or G and S units by *p*-hydroxycinnamoyl-CoA:shikimate/quinate *p*-hydroxycinnamoyl transferase (HCT), and (ii) coniferaldehyde, dedicated either to G lignin units by cinnamyl alcohol dehydrogenase (CAD) or to S lignin units by ferulate 5-hydroxylase (F5H). PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; C3H, *p*-coumarate 3-hydroxylase; CCoAOMT, caffeoyl-CoA *O*-methyltransferase. The usual carbon numbering of aromatic ring is given for cinnamic acid. R = shikimic or quinic acid.

the third chapter by Harrington *et al.*, together with novel tools to decipher genetic correlations between lignin-related genes.

Cytochrome P450 hydroxylases are key actors of the lignin pathway, which comprises three hydroxylation steps catalysed by cinnamate 4-hydroxylase, *p*-coumarate 3-hydroxylase and ferulate 5-hydroxylase (Fig. 1). In the past decade, the representation of the lignin pathway has changed considerably from discoveries about these apparently simple hydroxylation steps, as reviewed by Alber and Ehlting in the fourth chapter.

Lignin polymerization occurs via coupling of phenoxy radicals issued from the enzymatically driven oxidation of phenolic precursors, a mechanism evidenced in pioneering model studies of Freudenberg in the 1950s. *In vivo*, the involvement of peroxidases in the polymerization of lignin precursors is well established. In contrast, the role of plant laccases in lignin polymerization was poorly established until recent findings, as discussed by Berthet *et al.* in the fifth chapter.

Both the lignin biosynthetic pathway and the formation of secondary walls are positively or negatively regulated by a complex array of transcription factors, organized in a sophisticated hierarchical network. This novel aspect of lignification is the topic of the comprehensive and up-to-date review by Grima-Pettenati *et al.* in the sixth chapter.

Vascular plants respond to various stresses by the stimulation of the phenylpropanoid pathway, leading to various phenolics and lignins. Another fascinating aspect of these multifaceted polymers relies in the formation of stress lignins. Whereas their significance as barriers against pathogen attacks is documented, the literature data about the relationships of lignification to abiotic stresses are more sketchy. In the seventh chapter, Cabané *et al.* provide a comprehensive review of lignins formed in response to numerous abiotic stresses, their potential roles and the mechanisms underlying their biosynthesis.

Although lignins impart cell wall resistance against microbial attack, their biodegradation plays an important role in the carbon cycle. The eighth chapter, by Sigoillot *et al.*, displays the current understanding of fungal strategies for lignin degradation. The authors highlight the complexity of the required enzymatic cocktail made of various oxidases and auxiliary enzymes. They underline the importance of high-throughput phylogenetic tools to identify novel enzymes better suited for industrial uses and review recent progress in the production of efficient recombinant ligninases.

Plants have evolved the route to lignins as a strategy to colonize terrestrial ecosystems. Until recently, lignins were considered as the hallmark of tracheophytes, and syringyl S units were viewed as angiosperm specific, except in the *Selaginella* lycophyte and in some exceptional conifers (as reported in the 1971 *Lignins* textbook). This situation has been reassessed by the recent discovery of lignins (in trace amount) in a red alga and in some bryophytes, as well by the identification of the *Selaginella* enzyme dedicated to S units and distinct from that of angiosperms. In their cutting-edge review (ninth chapter), Prof. Ros Barceló and his coworkers provide an evolutionary view of how lignification emerged in different phylogenetic groups and by convergent pathways.

We express our gratitude to the contributing authors for their dedicated efforts in documenting the latest advances in their respective fields. In addition, we would like to thank those who spent hours reviewing each chapter.

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