



Harnessing lignin evolution for biotechnological applications

Hugues Renault, Daniele Werck-Reichhart, Jing-Ke Weng

► To cite this version:

Hugues Renault, Daniele Werck-Reichhart, Jing-Ke Weng. Harnessing lignin evolution for biotechnological applications. *Current Opinion in Biotechnology*, 2019, 56, pp.105-111. <10.1016/j.copbio.2018.10.011>. <hal-02438114>

HAL Id: hal-02438114

<https://hal.science/hal-02438114v1>

Submitted on 14 Jan 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



Harnessing lignin evolution for biotechnological applications

Hugues Renault¹, Danièle Werck-Reichhart¹ and
Jing-Ke Weng^{2,3}

Lignin evolved concomitantly with the rise of vascular plants on planet earth ~450 million years ago. Several iterations of exploiting ancestral phenylpropanoid metabolism for biopolymers occurred prior to lignin that facilitated early plants' adaptation to terrestrial environments. The first true lignin was constructed via oxidative coupling of a number of simple phenylpropanoid alcohols to form a sturdy polymer that supports long-distance water transport. This invention has directly contributed to the dominance of vascular plants in the Earth's flora, and has had a profound impact on the establishment of the rich terrestrial ecosystems as we know them today. Within vascular plants, new lignin traits continued to emerge with expanded biological functions pertinent to host fitness under complex environmental niches. Understanding the chemical and biochemical basis for lignin's evolution in diverse plants therefore offers new opportunities and tools for engineering desirable lignin traits in crops with economic significance.

Addresses

¹ Institute of Plant Molecular Biology, CNRS UPR 2357, University of Strasbourg, F-67000 Strasbourg, France

² Whitehead Institute for Biomedical Research, 455 Main Street, Cambridge, MA 02142, USA

³ Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Corresponding authors:

Renault, Hugues (renault@unistra.fr),

Werck-Reichhart, Danièle (werck@unistra.fr),

Weng, Jing-Ke (wengj@wi.mit.edu)

Current Opinion in Biotechnology 2019, **56**:105–111

This review comes from a themed issue on **Plant biotechnology**

Edited by **John Ralph** and **Wout Boerjan**

<https://doi.org/10.1016/j.copbio.2018.10.011>

0958-1669/© 2018 Elsevier Ltd. All rights reserved.

Introduction

Lignin is a class of 4-hydroxyphenylpropanoid-derived biopolymers uniquely and ubiquitously associated with vascular plants [1]. Lignin provides structural rigidity to the plant fibers and water-conducting xylem cells in the vasculature, and enables these specialized cells to withstand the negative pressure caused by surface tension when water is transported from roots to the distal parts of

the plants. It is thought that the emergence of lignin in early vascular plants around 450 million years ago propelled the rapid evolution of land plants, and directly contributed to their dominance of the terrestrial ecosystems. In addition to its primary role in strengthening vascular tissues, lignin also confers a remarkable physical and chemical barrier that shields important tissues of vascular plants from being consumed by the co-evolving terrestrial animals and microbes. For example, many seed plants evolved highly lignified seed shells to protect the vulnerable embryo encased within. Moreover, tremendous amounts of carbon fixation by early vascular plants in the form of lignin and a lag in the evolution of microbial enzymes that can efficiently degrade lignin are considered as major factors that led to the significant increase of the O₂/CO₂ ratio in the late Paleozoic era [2,3].

Lignin is mainly polymerized from three hydroxycinnamyl alcohols, *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, through radical coupling, to give *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin units, respectively [1]. Except for S lignin, which is taxonomically restricted to certain lineages, for example flowering plants, Selaginellaceae, and some species of ferns and gymnosperms, the H and G lignins are present in all the vascular plants [4,5]. Other less conserved monomers are also found as integral components of natural lignins or in mutant lignins resulting from engineered modifications. During lignin polymerization, monolignol radicals generated by peroxidases or laccases crosslink with the growing lignin polymer to form a polymer possessing a rich set of interunit linkages, a process dictated by the chemical identity of available monolignols and the local matrix environment [1]. Monomer composition and interunit linkage diversity of lignins vary greatly among different plants and/or different tissues types, and contribute to different physicochemical properties of the derived lignin polymers suited for their selected biological functions.

Lignin biosynthesis emerged from the ancestral general phenylpropanoid metabolism, which became established in early terrestrial plants when they migrated from water to land approximately 500 million years ago [5]. Several recent studies on phenylpropanoid metabolism in bryophytes, and lignin chemistry and biochemistry in diverse lineages of vascular plants, have shed new light on how lignin might have originated and subsequently diversified concomitantly with the proliferation of land plants. Here, we

provide an updated review on lignin evolution. We draw connections with potential biotechnological applications given our expanded understanding of lignin evolution.

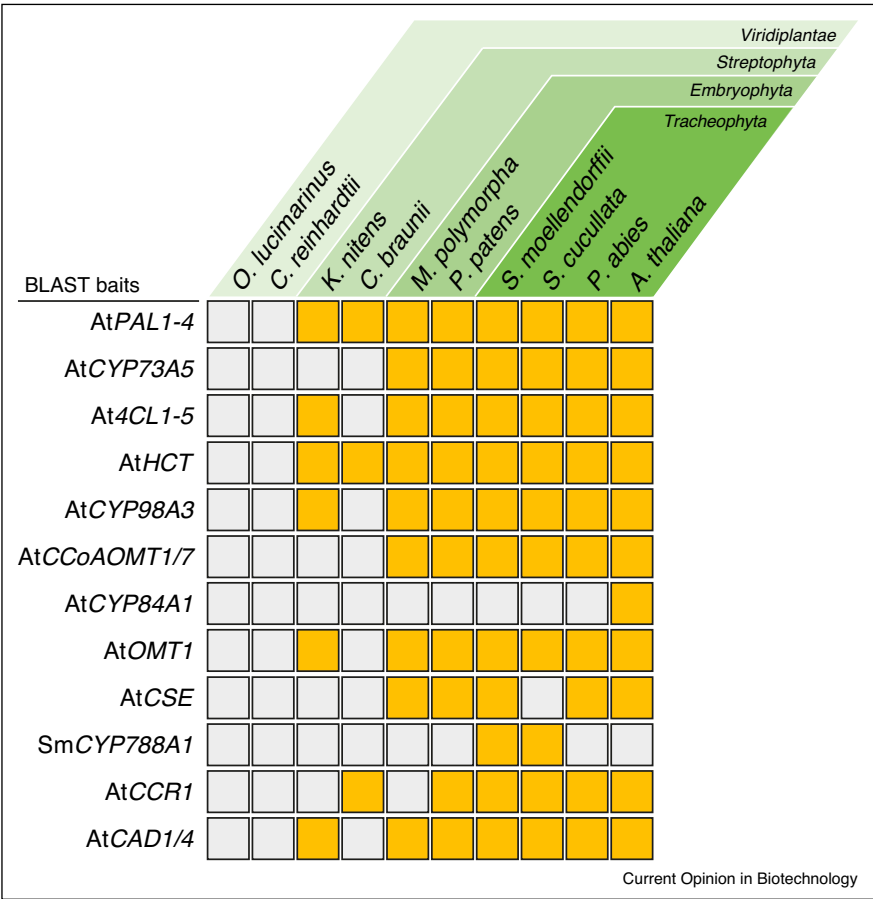
Early evolution of ‘lignin-like’ phenylpropanoids in non-vascular plants

Traces of tracheophyte-type lignin was reported in intertidal red alga *Calliarthron cheilosporioides* [6]. However, red algae diverged from the green lineage around 1.7 billion years ago and their genomes lack orthologs of land plant phenylpropanoid genes [7], suggesting either *C. cheilosporioides* acquired lignin biosynthesis through convergent evolution or the monolignols could be derived from terrestrial wood decay that entered the coastal ocean. Conversely, putative orthologs of several core phenylpropanoid genes are found in charophytes (Figure 1 and [8]), a group paraphyletic to land plants. It is therefore likely that the phenylpropanoid pathway emerged in these freshwater algae. Cell wall analyses in charophytes further provided evidence for this notion [9,10]. However, true lignin has

never been reported in any non-vascular green plant to date.

Studies of phenylpropanoid metabolism in basal land plants illuminate possible progenitor pathways of lignin biosynthesis. The model moss *Physcomitrella patens* contains significant amounts of caffeate-derived monomers in its cuticle [11]. The importance of these monomers was further highlighted by functional characterization of the CYP98 ortholog in *P. patens*, which catalyzes the phenolic ring *meta*-hydroxylation to yield caffeate derivatives [12]. The moss CYP98-null mutants exhibit gametophore growth defects associated with cutin deficiency. Unlike tracheophyte CYP98 orthologs which employs *p*-coumaroyl shikimate as its substrate, the *P. patens* CYP98 apparently uses *p*-coumaroyl threonate. Since threonate is a catabolite of ascorbate, this ‘pre-lignin’ pathway in *P. patens* seems to be associated with ascorbate metabolism. It is essential for cuticle formation and surface permeability control. The moss dual phenolic/aliphatic cuticle

Figure 1



Phylogenomics of lignin biosynthetic genes across the major green plant lineages. A reciprocal best hits strategy was implemented using BLASTp to determine the presence (yellow box) or absence (grey box) of putative orthologs of characterized lignin biosynthetic genes in fully sequenced plant genomes. At, *Arabidopsis thaliana*; Sm, *Selaginella moellendorffii*.

polymer displays commonalities shared among cutin, suberin, lignin and sporopollenin polymers found in tracheophytes. It may thus represent an ancestral iteration of land plant biopolymer evolution, which likely also supplied raw materials that later seeded lignin evolution (Figure 2).

Whether the phenolic-enriched cuticle polymer discovered in *P. patens* is widespread in other plants is yet to be examined. Biochemical characterization of additional CYP98 homologs from basal land plant lineages should provide more hints. A synchrotron X-ray analysis of the fossilized arborescent lycophytes, which are the major constituents of many Carboniferous coal deposits, supports the ancestral prevalence of an extracellular mixed polymer containing both phenolic and aliphatic components [13,14^{*}]. The *Lepidodendron* fossil samples analyzed in this study are scantily lignified, but with 55% of their biomass derived from periderm tissue. The fossilized tissue revealed a suberin-like chemistry with greater abundance of aliphatics than phenolics, comparable with but not identical to the bark of extant seed plant trees.

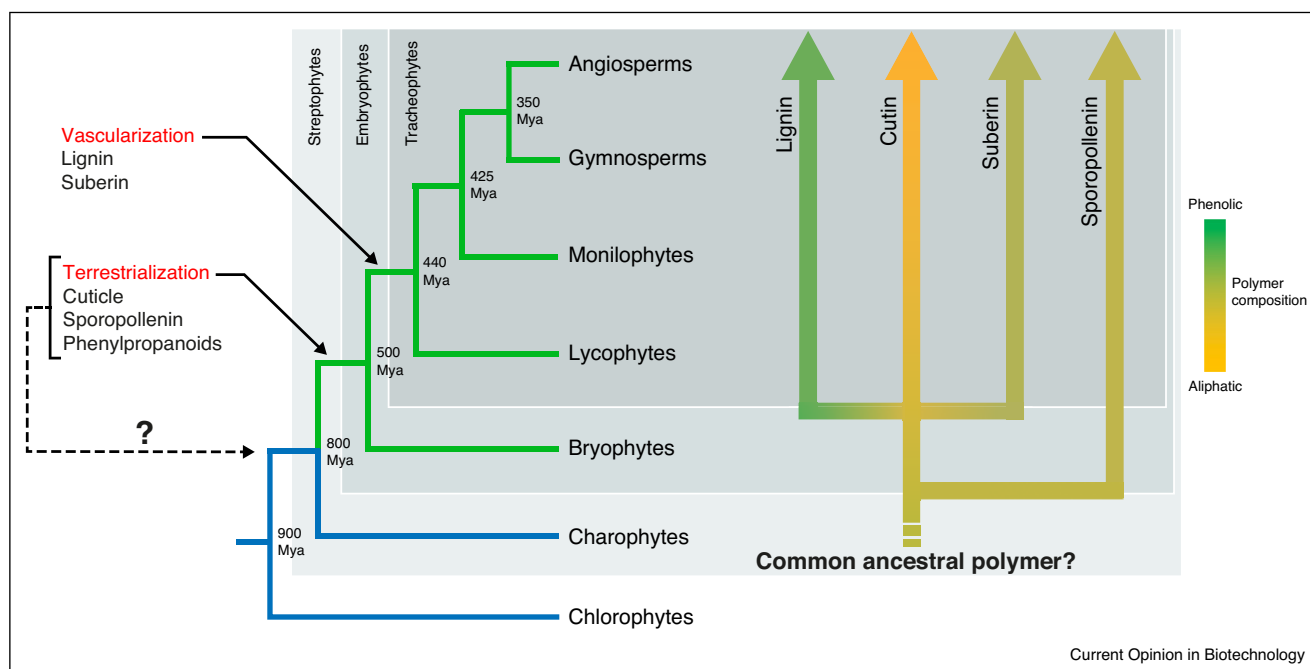
Variation of lignin chemistry in vascular plants

True lignin arose unequivocally with the emergence of vascular plants. Since then, lignin biosynthetic machinery has greatly diversified to yield a multitude of lignin chemotypes with specific functional implications. One

of the major taxonomic distribution patterns of lignin composition is the prevalent occurrence of S lignin in all angiosperms and the lycophytes under the *Selaginella* genus, and the general absence of S lignin in non-*Selaginella* lycophytes, most monilophytes, and gymnosperms (Figure 3a) [15]. As sinapyl alcohol cannot engage in β -5', 5-5', 4-*O*-5' linkages as can the H and G lignin monomers, S lignin is less condensed [1]. Studies of tissue-specific lignification across various plants showed that tracheids and vessel elements are generally G-lignin rich, whereas phloem and fiber cells contain more S lignin [16,17,18^{**}]. This general observation suggests that the ancestral function of G lignin is associated with reinforcing the cell wall of water-conducting tracheary element (TE)s, whereas S lignin, as a more derived trait, may serve alternative functions, such as defense.

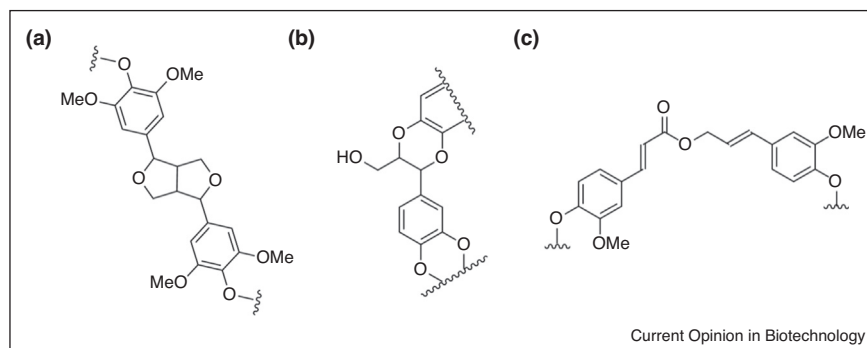
Certain industrial applications favor S-rich lignocellulosic biomass, because S lignin is less condensed than G lignin and therefore is associated with enhanced enzymatic and chemical digestibility. Genetic engineering of the S-lignin biosynthetic branch, with its *ferulate 5-hydroxylase* (*F5H*) and *caffeic acid O-methyltransferase* (*COMT*) genes, has proven to be effective to increase S lignin content and improve cell wall digestibility in multiple angiosperm species [15,19–21]. Lignin of conifers is generally devoid of S units. Using a transformable TE system of pine, Wagner *et al.* recently demonstrated the feasibility of

Figure 2



The timeline depicting the evolution of lignin and possible relationships with other hydrophobic apoplastic polymers in the green lineage. Approximate timescales at tree nodes were determined according to Ref. [50].

Figure 3



Evolutionarily new lignin chemotypes with potential biotechnological applications.

(a) β-β-Coupled syringyl (S) lignin units ubiquitously found in angiosperms and lycophytes under the family Selaginellaceae. Although this unit is considered to be a more condensed unit, S lignin is otherwise less condensed compared to G lignin. **(b)** Caffeyl (C) lignin units crosslinked through benzodioxane moiety found in seed lignins of certain angiosperm species. Lignin composed of pure C units possesses physicochemical properties distinct from other lignin types. **(c)** Ferulate (FA) ester units found in various angiosperm species, including Chinese angelica, kenaf, and balsa. Incorporation of monolignol ferulate conjugates into lignin yields engineered lignin polymers with hydrolyzable esters in their backbones.

engineering S lignin into conifers [22]. The authors showed that transgenic expression of the sweetgum *F5H* alone was sufficient to result in S lignin production, whereas co-expression of *F5H* with *COMT* led to more S lignin production [22]. Besides the well-established case of parallel evolution of S lignin in Selaginellaceae [23], several ferns, such as *Ceratopteris cornuta* and *Dennstaedtia bipinnata*, and gymnosperms, including the yew plum pine *Podocarpus macrophyllus* and all three extant families of gnetophyta, were found to contain S lignin [4,24]. Although yet to be identified, the S lignin biosynthetic genes from these non-angiosperm S-lignin-producing plants will be valuable tools for engineering S lignin in economically important gymnosperms.

Seed plants first appeared in the Late Devonian (~370 million years ago) and have dominated the Earth's flora since then. Many seeds feature heavily lignified seed coats, suggesting that lignin was co-opted for the purpose of seed protection. A recent study of the seed lignin of monocot vanilla orchid (*Vanilla planifolia*) revealed a new homogeneous lignin polymer (C lignin) entirely derived from caffeyl alcohol crosslinked through benzodioxane units (Figure 3b), a linkage previously only described in mutant lignin composed of 5-hydroxyguaiacyl (5-OH-G) units [25^{••}]. A subsequent study of seed lignins prepared from 130 different dicot cactus species further established that C lignin is the dominant lignin chemotype of cactus seed coat, and some species also contain natural 5H lignin derived from the 5-OH-G unit [26]. Both C and 5H lignins are crosslinked through the distinct benzodioxane moiety, resulting in different physicochemical properties than G and S lignins. As C lignin was found sporadically in seeds from both monocots and dicots, it likely evolved

multiple times and bestows unique selective advantages to certain seed plants [27[•]]. C and 5H lignins can in principle be engineered into target plants by repressing the two lignin biosynthetic methyltransferases, *caffeoyl CoA-3-O-methyltransferase* (CCoAOMT) and *COMT*, which may find use for specific biomass engineering. Indeed, simultaneously overexpressing *F5H* and downregulating *COMT* results in transgenic lignin significantly enriched with 5H units [28,29].

Canonical lignins contain predominant C–O–C and C–C bonds, which present significant difficulty for chemical or enzymatic degradation. Incorporation of ester bonds into lignin therefore can potentially confer lignin with easily-hydrolyzable properties. Grass lignins are naturally acylated with *p*-coumarates [30]. However, during lignin polymerization, monolignol-*p*-coumarate (ML-*p*-CA) conjugates prefer radical transfer over radical coupling. As a result, ML-*p*-CAs exist mostly as peripheral units of the lignin polymer [31]. Karlen *et al.* surveyed the presence of lignins derived from monolignol-ferulates (ML-FA) in a wide selection of gymnosperm and angiosperm species, and showed that ML-FA is generally absent in gymnosperms, but occurs as an integral unit of lignin in various angiosperm species, such as Chinese angelica (*Angelica sinensis*), kenaf (*Hibiscus cannabinus*), and balsa (*Ochroma pyramidale*) (Figure 3c) [32[•]], as well as all grasses. Unlike ML-*p*-CA, ML-FA contains an additional methoxyl group, and therefore is more prone to radical coupling with normal monolignol radicals than ML-*p*-CA during polymerization. Wilkerson *et al.* identified a BAHD acyltransferase from *A. sinensis* that functions as a feruloyl-coenzyme A (CoA) monolignol transferase (FMT) to catalyze the formation of ML-FA [33].

Overexpression of *FMT* in poplar led to the incorporation of ester bonds in the transgenic lignin backbone and much improved cell wall digestibility under mild alkaline pretreatment condition [33].

Biochemical basis for lignin pathway evolution

Gene duplication followed by subfunctionalization or neo-functionalization can partly explain the divergent evolution of lignin/cell wall chemistry. For example, a duplication event of the ancestral gene encoding phenylalanine ammonia-lyase (PAL) occurred in monocots after they diverged from dicots, which led to the emergence of a bifunctional Phe and Tyr ammonia-lyase (PTAL). This enzyme is encoded by a single gene in the model grass *Brachypodium distachyon*. Using a combination of isotope precursor feeding and RNAi suppression, Barros *et al.* demonstrated that about half of the lignin in *B. distachyon* was derived from tyrosine via PTAL [34[•]]. Tyrosine is preferentially traced into S lignin and cell-wall-bound *p*-coumarate (but not ferulate), suggesting the existence of parallel lignin biosynthetic pathways organized at tissue and/or cellular levels. The presence of an active-site His residue anchoring the Tyr phenol ring was found to be the key structural feature that converts PAL to PTAL [35]. Early duplication events have occurred independently in other land plant lineages [35–37], suggesting that enzymes with dual PAL/TAL activities may also exist beyond grasses.

The orthology of *cinnamate 4-hydroxylase* (*C4H*) is well preserved in most land plant genomes, implying *C4H* gene is under strong negative selection [38[•]]. From the two successful *C4H* duplications, one occurred in early seed plants prior to the divergence of gymnosperms and angiosperms and the second in commelinid monocots, only the former has been documented to date [38[•]]. The resulting *C4H* paralog is retained in Taxaceae (gymnosperms) and most angiosperms (except Brassicaceae), and exhibits puzzling structural divergences from the canonical *C4H*. Although the role of this newly derived *C4H* paralog is yet to be determined in its native host species, it encodes a functional *C4H* enzyme that is capable of complementing the *Arabidopsis c4h* null mutants [38[•]]. Homology modelling revealed a conserved insertion of several amino acids, in a region involved in enzyme interaction with its electron donor. In addition, instead of the conserved single *N*-terminal-membrane-spanning helix that anchors the canonical *C4H* to the endoplasmic reticulum (ER) membrane facing cytosol, the derived *C4H* paralog features an elongated and more variable *N*-terminus that forms a double-spanning hairpin, allowing orientation of *C4H* to the ER lumen. As *C4H* is proposed to anchor lignin biosynthetic metabolon to ER, the presence of a second *C4H* with altered membrane topology implies the possible existence of independently assembled multi-enzyme complexes for specific aspects of phenylpropanoid metabolism. The

presence of lignin biosynthetic metabolons is recently substantiated by the discovery of several membrane steroid-binding proteins (MSBPs), which serve as scaffold proteins to assemble monolignol P450s to ER [39[•]].

The expansion of the 4-coumarate-CoA ligase (4CL) family in most plant genomes further supports the parallel pathway hypothesis. A recent phylogenetic analysis based on 194 4CL sequences collected from a broad spectrum of land plants revealed one major 4CL duplication event occurred in seed plants prior to the split of gymnosperms and angiosperms, resulting in a separate monophyletic clade [40[•]]. Functional analysis of the four 4CL paralogs present in *Arabidopsis thaliana* showed that the representative paralog of the most early diverged clade has acquired a distinct role in flavonoid biosynthesis, while also contributing to sinapoyl malate biosynthesis [40[•]]. The other three 4CL paralogs appear to have subfunctionalized to play different physiological roles in various growth and developmental processes involving lignification. The existence of parallel lignin biosynthetic pathways at cellular and tissue levels provides opportunities for future precision engineering of lignin traits in target crops.

Genes controlling the entry point of phenylpropanoid pathway to lignification appear to be highly conserved. Both *hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl-transferase* (*HCT*) and *p-coumaroyl shikimate 3'-hydroxylase* (*C3'H*) orthologs are present in all land plants, often as single-copy genes, and their emergence preceded the occurrence of lignin (Figure 1). For *HCT*, one major gene duplication occurred in dicots, giving rise to a clade of neofunctionalized quinate-acylating enzymes responsible for the biosynthesis of chlorogenic acids [41]. Another gene duplication event of *HCT* occurred in the common ancestor of Lamiaceae that led to the birth of rosmarinic acid synthase (RAS) and the biosynthesis of rosmarinic acid [42]. Similarly, recently derived *C3'H* paralogs in certain plants have acquired new phenolic *m*-hydroxylase activities that prefer alternative *p*-coumaroyl conjugates as substrates [43,44]. To date, most described gene duplication cases involving *HCT* and *C3'H* led to the production of new soluble phenolic esters and amides [41,43,45]. The only exception is the duplication of both *HCT* and *C3'H* genes to form, in Brassicaceae, an alternative pathway leading to feruloyl-conjugated and sinapoyl-conjugated phenolamides that contribute to the pollen coat and the strengthening of pollen wall [46,47[•],48]. These observations suggest that promiscuous activities of conserved lignin biosynthetic enzymes likely sowed the seeds for new phenylpropanoid metabolic traits. Enzymes evolved through such mechanism can in turn be employed to engineer desirable lignin traits, such as the aforementioned FMT [33]. Moreover, enzyme promiscuity has also inspired new strategies to manipulate lignin biosynthesis by engineering the production of endogenous acyl acceptor molecules that compete for HCT activity *in vivo* [49[•]].

Perspective

Nature has explored countless experiments with lignification over the past 450 million years of vascular plant evolution. Studies of other related biopolymer biosynthetic pathways as well as phenylpropanoid metabolism in several nonvascular green plant lineages further provide valuable comparative perspectives on how lignification evolved in the first place. Understanding lignin evolution will continue to yield new approaches for genetic engineering of lignin biosynthesis. Several key questions regarding lignin evolution remain to be resolved in the coming years. For example, what are the functional significances of various lignin chemotypes found in diverse plants and tissue types? How are parallel lignification pathways organized and regulated at biochemical, cellular and tissue levels? Was coupling phenylpropanoid metabolism with the shikimate pathway via HCT a key event during lignin evolution? Answering these questions will further enable us to craft 'designer' lignins in future biotech crops.

Conflict of interest statement

Nothing declared.

Acknowledgements

HR is supported by the initiative of excellence IDEX-unistra (ANR-10-IDEX-0002-02) under the French national program 'Investment for the future'. This work was supported by grants from the Searle Scholars Program (15-SSP-162) and the National Science Foundation (1709616) to JKW.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Ralph J, Lundquist K, Brunow G, Lu F, Kim H, Schatz PF, Marita JM, Hatfield RD, Ralph SA, Christensen JH *et al.*: **Lignins: natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids**. *Phytochem Rev* 2004, **3**:29-60.
2. Berner RA: **Isotope fractionation and atmospheric oxygen: implications for phanerozoic O₂ evolution**. *Science* 2000, **287**:1630-1633.
3. Berner RA: **Paleozoic atmospheric CO₂: importance of solar radiation and plant evolution**. *Science* 1993, **261**:68-70.
4. Espiñeira JM, Novo Uzal E, Gómez Ros LV, Carrión JS, Merino F, Ros Barceló A, Pomar F: **Distribution of lignin monomers and the evolution of lignification among lower plants**. *Plant Biol* 2011, **13**:59-68.
5. Weng J-K, Chapple C: **The origin and evolution of lignin biosynthesis**. *New Phytol* 2010, **187**:273-285.
6. Martone PT, Estevez JM, Lu F, Ruel K, Denny MW, Somerville C, Ralph J: **Discovery of lignin in seaweed reveals convergent evolution of cell-wall architecture**. *Curr Biol* 2009, **19**:169-175.
7. Brawley SH, Blouin NA, Ficko-Blean E, Wheeler GL, Lohr M, Goodson HV, Jenkins JW, Blaby-Haas CE, Helliwell KE, Chan CX *et al.*: **Insights into the red algae and eukaryotic evolution from the genome of *Porphyra umbilicalis* (Bangioophyceae, Rhodophyta)**. *Proc Natl Acad Sci U S A* 2017, **114**:E6361-E6370.
8. de Vries J, de Vries S, Slamovits CH, Rose LE, Archibald JM: **How embryophytic is the biosynthesis of phenylpropanoids and their derivatives in streptophyte algae?** *Plant Cell Physiol* 2017, **58**:934-945.
9. Sørensen I, Pettolino FA, Bacic A, Ralph J, Lu F, O'Neill MA, Fei Z, Rose JKC, Domozych DS, Willats WGT: **The charophycean green algae provide insights into the early origins of plant cell walls**. *Plant J* 2011, **68**:201-211.
10. Delwiche CF, Graham LE, Thomson N: **Lignin-like compounds and sporopollenin coelochaete, an algal model for land plant ancestry**. *Science* 1989, **245**:399-401.
11. Buda GJ, Barnes WJ, Fich EA, Park S, Yeats TH, Zhao L, Domozych DS, Rose JKC: **An ATP binding cassette transporter is required for cuticular wax deposition and desiccation tolerance in the moss *Physcomitrella patens***. *Plant Cell* 2013, **25**:4000-4013.
- First report of the presence of a large proportion of phenolic units in the cuticle of the model moss *Physcomitrella patens*.
12. Renault H, Alber A, Horst NA, Basilio Lopes A, Fich EA, Kriegshauser L, Wiedemann G, Ullmann P, Herrgott L, Erhardt M *et al.*: **A phenol-enriched cuticle is ancestral to lignin evolution in land plants**. *Nat Commun* 2017, **8**:14713.
- First hint to potential ancestral role of the pre-lignin pathway in bryophytes. Reveals essential role of caffeate units for the formation of moss cuticle, gametophore development and permeability. Also suggests that *meta*-hydroxylation of *p*-coumaric in *P. patens* is likely to occur on threonate instead of shikimate ester.
13. Nelsen MP, DiMichele WA, Peters SE, Boyce CK: **Delayed fungal evolution did not cause the Paleozoic peak in coal production**. *Proc Natl Acad Sci U S A* 2016, **113**:2442-2447.
14. Boyce CK, Kevin Boyce C, Abrecht M, Zhou D, Gilbert PUPA: **X-ray photoelectron emission spectromicroscopic analysis of arborescent lycopsid cell wall composition and Carboniferous coal ball preservation**. *Int J Coal Geol* 2010, **83**:146-153.
- Interesting tentative analysis of *Lepidodendron* (lycopsid tree canopy-dominants in coal swamp forests) bark in fossil coal deposits from the Late Paleozoic. Lycopsids constitute 60–80% of most Carboniferous coals, and periderm alone up to 20–56% of these coals.
15. Weng J-K, Li X, Stout J, Chapple C: **Independent origins of syringyl lignin in vascular plants**. *Proc Natl Acad Sci U S A* 2008, **105**:7887-7892.
16. Lourenço A, Rencoret J, Chemetova C, Gominho J, Gutiérrez A, del Río JC, Pereira H: **Lignin composition and structure differs between xylem, phloem and phellem in *Quercus suber* L.** *Front Plant Sci* 2016, **7**.
17. Barros J, Serk H, Granlund I, Pesquet E: **The cell biology of lignification in higher plants**. *Ann Bot* 2015, **115**:1053-1074.
18. Weng J-K, Akiyama T, Bonawitz ND, Li X, Ralph J, Chapple C: **Convergent evolution of syringyl lignin biosynthesis via distinct pathways in the lycophyte *Selaginella* and flowering plants**. *Plant Cell* 2010, **22**:1033-1045.
- Together with [23], characterization of the lycophyte-specific pathway which led by convergent evolution to the formation of S-lignin in *Selaginellaceae*. Two clustered enzymes, a cytochrome P450 and an O-methyltransferase are both bifunctional to decorate the phenolic ring in its 3- and 5-positions.
19. Meyer K, Shirley AM, Cusumano JC, Bell-Lelong DA, Chapple C: **Lignin monomer composition is determined by the expression of a cytochrome P450-dependent monooxygenase in *Arabidopsis***. *Proc Natl Acad Sci U S A* 1998, **95**:6619-6623.
20. Franke R, McMichael CM, Meyer K, Shirley AM, Cusumano JC, Chapple C: **Modified lignin in tobacco and poplar plants over-expressing the *Arabidopsis* gene encoding ferulate 5-hydroxylase**. *Plant J* 2000, **22**:223-234.
21. Stewart JJ, Akiyama T, Chapple C, Ralph J, Mansfield SD: **The effects on lignin structure of overexpression of ferulate 5-hydroxylase in hybrid poplar**. *Plant Physiol* 2009, **150**:621-635.
22. Wagner A, Tobimatsu Y, Phillips L, Flint H, Geddes B, Lu F, Ralph J: **Syringyl lignin production in conifers: proof of concept in a Pine tracheary element system**. *Proc Natl Acad Sci USA* 2015, **112**:6218-6223.
23. Weng J-K, Akiyama T, Ralph J, Chapple C: **Independent recruitment of an O-methyltransferase for syringyl lignin**

- biosynthesis in *Selaginella moellendorffii*.** *Plant Cell* 2011, **23**:2708-2724.
24. Weng J-K, Banks JA, Chappel C: **Parallels in lignin biosynthesis.** *Commun Integr Biol* 2008, **1**:20-22.
 25. Chen F, Tobimatsu Y, Havkin-Frenkel D, Dixon RA, Ralph J: **A polymer of caffeoyl alcohol in plant seeds.** *Proc Natl Acad Sci U S A* 2012, **109**:1772-1777.
- First demonstration that seeds of some plants like vanilla or Cactaceae accumulated so-called C-lignin (formed of polymerized caffeoyl units). Ref. [26] extends this investigation to determine distribution of C-lignin within Cactaceae and to show that C-lignin is not found within the most ancestral subfamily of Opuntioideae, but is common in Cactoideae.
26. Chen F, Tobimatsu Y, Jackson L, Nakashima J, Ralph J, Dixon RA: **Novel seed coat lignins in the Cactaceae: structure, distribution and implications for the evolution of lignin diversity.** *Plant J* 2013, **73**:201-211.
 27. Tobimatsu Y, Chen F, Nakashima J, Escamilla-Treviño LL, Jackson L, Dixon RA, Ralph J: **Coexistence but independent biosynthesis of catechyl and guaiacyl/syringyl lignin polymers in seed coats.** *Plant Cell* 2013, **25**:2587-2600.
- Shows that C-lignin can coexist with G/S lignin, deposited as consecutive layers in the same seed testa.
28. Vanholme R, Ralph J, Akiyama T, Lu F, Pazo JR, Kim H, Christensen JH, Van Reusel B, Storme V, De Rycke R *et al.*: **Engineering traditional monolignols out of lignin by concomitant up-regulation of F5H1 and down-regulation of COMT in Arabidopsis.** *Plant J* 2010, **64**:885-897.
 29. Weng J-K, Mo H, Chapple C: **Over-expression of F5H in COMT-deficient Arabidopsis leads to enrichment of an unusual lignin and disruption of pollen wall formation.** *Plant J* 2010, **64**:898-911.
 30. Hatfield RD, Marita JM, Frost K, Grabber J, Ralph J, Lu F, Kim H: **Grass lignin acylation: p-coumaroyl transferase activity and cell wall characteristics of C3 and C4 grasses.** *Planta* 2009, **229**:1253-1267.
 31. Ralph J: **Hydroxycinnamates in lignification.** *Phytochem Rev* 2009, **9**:65-83.
 32. Karlen SD, Zhang C, Peck ML, Smith RA, Padmakshan D, Helmich KE, Free HCA, Lee S, Smith BG, Lu F *et al.*: **Monolignol ferulate conjugates are naturally incorporated into plant lignins.** *Sci Adv* 2016, **2**:e1600393.
- Interesting tentative correlation of the presence of zip lignin (incorporating ferulate conjugates monolignols) with a specific clade of BAHD transferases. Demonstrates repeated and convergent evolution of the trait, suggesting that it provides a punctual selective advantage.
33. Wilkerson CG, Mansfield SD, Lu F, Withers S, Park J-Y, Karlen SD, Gonzales-Vigil E, Padmakshan D, Unda F, Rencoret J *et al.*: **Monolignol ferulate transferase introduces chemically labile linkages into the lignin backbone.** *Science* 2014, **344**:90-93.
 34. Barros J, Serrani-Yarce JC, Chen F, Baxter D, Venables BJ, Dixon RA: **Role of bifunctional ammonia-lyase in grass cell wall biosynthesis.** *Nat Plants* 2016, **2**:16050.
- A very interesting paper allying a diversity of approaches to demonstrate the importance of PTAL (a PAL with tyrosine ammonia-lyase activity) in the formation of S-lignin and wall-bound p-coumarate biosynthesis in the model monocot *Brachypodium distachyon*. Provides an essential basis to further investigate the complexity of the phenolic pathway in monocots.
35. Jun S-Y, Sattler SA, Cortez GS, Vermerris W, Sattler SE, Kang C: **Biochemical and structural analysis of substrate specificity of a phenylalanine ammonia-lyase.** *Plant Physiol* 2018, **176**:1452-1468.
 36. Bagal UR, Leebens-Mack JH, Lorenz WW, Dean JFD: **The phenylalanine ammonia lyase (PAL) gene family shows a gymnosperm-specific lineage.** *BMC Genomics* 2012, **13**:S1.
 37. Wu Z, Gui S, Wang S, Ding Y: **Molecular evolution and functional characterisation of an ancient phenylalanine ammonia-lyase gene (NnPAL1) from *Nelumbo nucifera*: novel insight into the evolution of the PAL family in angiosperms.** *BMC Evol Biol* 2014, **14**:100.
 38. Renault H, De Marothy M, Jonasson G, Lara P, Nelson DR, Nilsson I, André F, von Heijne G, Werck-Reichhart D: **Gene duplication leads to altered membrane topology of a cytochrome P450 enzyme in seed plants.** *Mol Biol Evol* 2017, **34**:2041-2056.
- Reveals a conserved C4H gene duplication occurring upon emergence of seed plants. Demonstrates that resulting enzymes maintain efficient C4H activity, but that one of them evolved altered membrane topology and is likely to receive electrons from a different redox partner. Overall, suggests so far overlooked complexity in the phenylpropanoid pathway.
39. Gou M, Ran X, Martin DW, C-J Liu: **The scaffold proteins of lignin biosynthetic cytochrome P450 enzymes.** *Nat Plants* 2018, **4**:299-310.
- First demonstration of the essential role of membrane steroid-binding proteins as structural components required to physically organize the monolignol pathway. Two paralogs seem to contribute to lignin synthesis in *Arabidopsis thaliana*. Homologs are present in many plant species.
40. Li Y, Kim JI, Pysh L, Chapple C: **Four isoforms of Arabidopsis 4-coumarate:CoA ligase have overlapping yet distinct roles in phenylpropanoid metabolism.** *Plant Physiol* 2015, **169**:2409-2421.
- Most plants feature several 4CL genes. This paper is the first attempt at resolving their specific roles and demonstrates specialized but overlapping functions
41. Sonnante G, D'Amore R, Blanco E, Pierri CL, De Palma M, Luo J, Tucci M, Martin C: **Novel hydroxycinnamoyl-coenzyme A quinate transferase genes from artichoke are involved in the synthesis of chlorogenic acid.** *Plant Physiol* 2010, **153**:1224-1238.
 42. Berger A, Meinhard J, Petersen M: **Rosmarinic acid synthase is a new member of the superfamily of BAHD acyltransferases.** *Planta* 2006, **224**:1503-1510.
 43. Eberle D, Ullmann P, Werck-Reichhart D, Petersen M: **cDNA cloning and functional characterisation of CYP98A and NADPH:cytochrome P450 reductase from *Coleus blumei* involved in rosmarinic acid biosynthesis.** *Plant Mol Biol* 2009, **69**:239-253.
 44. Matsuno M, Compagnon V, Schoch GA, Schmitt M, Debayle D, Bassard J-E, Pollet B, Hehn A, Heintz D, Ullmann P *et al.*: **Evolution of a novel phenolic pathway for pollen development.** *Science* 2009, **325**:1688-1692.
 45. Sander M, Petersen M: **Distinct substrate specificities and unusual substrate flexibilities of two hydroxycinnamoyltransferases, rosmarinic acid synthase and hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl-transferase, from *Coleus blumei* Benth.** *Planta* 2011, **233**:1157-1171.
 46. Grienemberger E, Besseau S, Geoffroy P, Debayle D, Heintz D, Lapiere C, Pollet B, Heintz T, Legrand M: **A BAHD acyltransferase is expressed in the tapetum of Arabidopsis anthers and is involved in the synthesis of hydroxycinnamoyl spermidines.** *Plant J* 2009, **58**:246-259.
 47. Liu Z, Tavares R, Forsythe ES, André F, Lugan R, Jonasson G, Boutet-Mercey S, Tohge T, Beilstein MA, Werck-Reichhart D *et al.*: **Evolutionary interplay between sister cytochrome P450 genes shapes plasticity in plant metabolism.** *Nat Commun* 2016, **7**:13026.
- Together with [44,46,48], illustrates the common features and potential cross-talk between lignin and sporopollenin metabolisms.
48. Xu J, Ding Z, Vizcay-Barrena G, Shi J, Liang W, Yuan Z, Werck-Reichhart D, Schreiber L, Wilson ZA, Zhang D: **ABORTED MICROSPORES acts as a master regulator of pollen wall formation in Arabidopsis.** *Plant Cell* 2014, **26**:1544-1556.
 49. Eudes A, Pereira JH, Yogiswara S, Wang G, Teixeira Benites V, Baidoo EEK, Lee TS, Adams PD, Keasling JD, Loqué D: **Exploiting the substrate promiscuity of hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase to reduce lignin.** *Plant Cell Physiol* 2016, **57**:568-579.
- Evolution-inspired attempt to exploit enzyme promiscuity to engineer lignin metabolism.
50. Morris JL, Puttick MN, Clark JW, Edwards D, Kenrick P, Pressel S, Wellman CH, Yang Z, Schneider H, Donoghue PCJ: **The timescale of early land plant evolution.** *Proc Natl Acad Sci U S A* 2018, **115**:E2274-E2283.