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Transcriptional networks for lignin biosynthesis: more complex than we thought?

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Lignin is an aromatic heteropolymer and the second most abundant plant biopolymer after cellulose. It is deposited mostly in the secondary cell walls of vascular plants and is essential for water transport, mechanical support and for plant pathogen defense. Lignin biosynthesis is a highly energy-consuming and irreversible process that responds to many developmental and environmental cues, including light, sugar content, circadian clock, plant hormones and wounding. During the past decade, many transcription factors involved in lignin biosynthesis have been identified and characterized. In this review, we assess how these transcriptional activators and repressors modulate lignin biosynthesis, and discuss crosstalk between the lignin biosynthesis pathway and other physiological processes.

Most genes of lignin biosynthesis are coregulated through AC-rich elements

Lignin is a phenylpropanoid-derived polymer found in specific cell types of vascular plants, particularly those with secondarily thickened cell walls involved in the transport of water or provision of mechanical strength. It is derived from three basic subunits; *p*-coumaryl, coniferyl and sinapyl alcohol. These monomers are known as the *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) monolignols when they are incorporated into the lignin polymer [1]. Monolignols are synthesized through the phenylpropanoid pathway, which is shared by other important secondary metabolites, such as flavonoids and coumarins.

Identification of *cis*-regulatory elements in most of the monolignol biosynthesis pathway genes has paved the way towards understanding the transcriptional regulation of lignin biosynthesis. Detailed promoter and electrophoretic mobility shift assay (EMSA) analysis has revealed that the AC-rich elements corresponding to the MYB transcription factor-binding motif are necessary for coordinated monolignol pathway gene activation [2–5]. AC elements are present in the promoters of most monolignol pathway genes, including *PAL* (L-phenylalanine ammonia-lyase), *4CL* (4-hydroxycinnamate: CoA ligase), *C3'H* (*p*-coumaroylshikimate 3′-hydroxylase), *CCoAOMT* (caffeoyl CoA 3-*O*-methyltransferase), *CCR* (cinnamoyl CoA reductase) and *CAD* (cinnamyl alcohol dehydrogenase) (Figure 1) [6]. Because the *C4H* (cinnamate 4-hydroxylase) and *COMT* (caffeic acid 3-*O*-methyltransferase) genes are also directly regulated by a lignin-specific MYB transcription factor, AtMYB58 (Figure 2), their regulatory regions might contain more degenerate AC elements [3]. However, the gene encoding F5H (ferulic acid 5-hydroxylase) (Figure 1), which is specifically involved in S-lignin biosynthesis, neither contains AC-rich elements nor is regulated by the lignin-specific transcription factors MYB58 and MYB63 [3]. Instead, it is directly regulated by a secondary cell wall master switch, NST3 (SND1), which itself activates MYB58 and MYB63 [7].

Transcriptional activators of lignin biosynthesis

Most lignin activators characterized to date are from the MYB family. PtMYB1, PtMYB4 and PtMYB8 from *Pinus taeda* (Table 1) [2,8,9], EgMYB2 from *Eucalyptus gunnii* (Table 1) [10,11] and PtrMYB3 and PtrMYB20 from *Populus trichocarpa* (Table 1) [12] all act to upregulate the monolignol pathway. MYB46, and its later-discovered homolog MYB83, are not only lignin pathway regulators, but also redundantly activate the entire process of secondary cell wall formation [13,14]. This is also the case for EgMYB2, PtMYB4, PtMYB8, PtrMYB3 and PtrMYB20, which are homologs of AtMYB46 [8]. The first identified true lignin-specific transcription factors were *Arabidopsis* (*Arabidopsis thaliana*) AtMYB85, AtMYB58 and AtMYB63 (Figure 2) [3,15]. The earlier identified PtMYB1 is a homolog of AtMYB85 [8]. Unlike the other MYB activators outlined above, which cause ectopic activation of the entire secondary cell wall program when overexpressed, these lignin-specific transcription factors only affect lignin biosynthesis [3,15]. Most monolignol genes are directly regulated by AtMYB58 through AC elements, except for *F5H* [3]. Thus, regulation by lignin activators is global rather than specific for certain pathway genes.

AtMYB61 causes ectopic lignification when overexpressed, and phenocopies a dark-photomorphogenic mutant, *de-etiolated 3* (*det3*). *DET3* encodes the C-subunits of the V-type ATPase. AtMYB61 is overexpressed in the *det3* mutant background, and suppression of AtMYB61 in this mutant restores the *det3* mutant phenotypes, including ectopic lignification and dark-photomorphogenic phenotypes [16]. There is evidence that AtMYB61 also controls stomatal aperture in *Arabidopsis* [17] and seed coat mucilage deposition [18].

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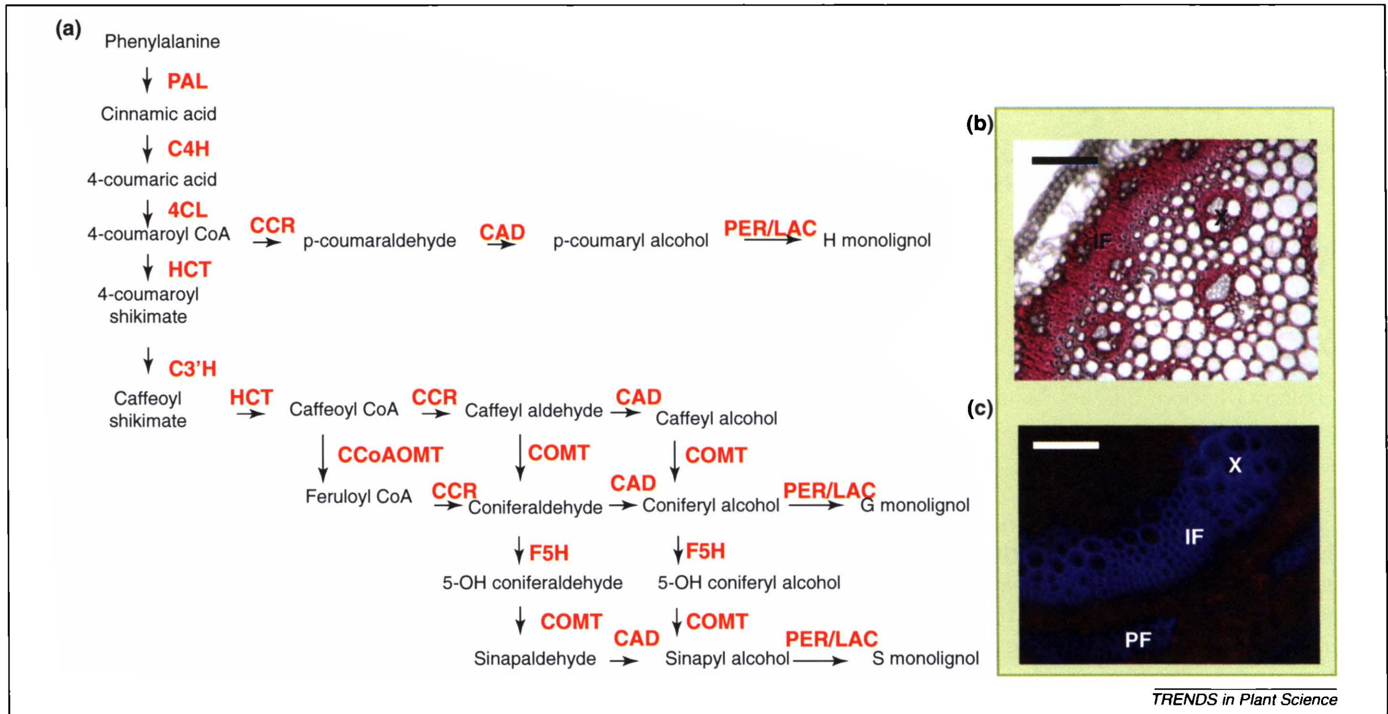

Figure 1. The monolignol biosynthetic pathway and typical lignin distribution in a monocot (Switchgrass) and dicot (*Medicago truncatula*). **(a)** The pathway to monolignols. The enzymes are as follows: PAL; C4H; 4CL; HCT; C3'H; CCoAOMT; CCR; CAD; F5H; COMT; peroxidase (PER); and laccase (LAC). **(b)** Phloroglucinol staining of lignin in a stem cross-section of switchgrass. **(c)** Lignin deposition visualized by UV autofluorescence in a stem cross-section of *Medicago truncatula*. Abbreviations: IF, interfascicular fibers; PF, phloem fibers; X, xylem elements. Scale bar = 100 mm. Reproduced, with permission, from Chunxiang Fu, Noble Foundation **(b)**.

Organ identity genes
MYB26
NST3
NST1
NST2
Cellulose xylan
MYB46
MYB83
Light, sucrose, wounding
Auxin
MYB58
MYB63
MYB85
MYB4
MYB32
BP
MYB75
F5H
AC element
Lignin biosynthesis genes
Anthocyanin
Lignin
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Figure 2. Current model of the transcriptional network of secondary cell wall biosynthesis in *Arabidopsis*. Both NST1/2/3 and MYB46/MYB83 can activate the entire secondary cell wall biosynthesis. F5H is regulated by NST3, whereas other lignin genes are regulated by MYB58/63/85 through the AC element. NSTs are downstream of organ identity genes. MYB4 is self-downregulated and is upregulated by light, wounding and sucrose. Auxin induces MYB32 expression but represses BP. Arrows indicate activation and the straight-line-end bars indicate inhibition.

Table 1. Transcription factors in lignin regulatory networks

Gene name	Species	Ortholog in *Arabidopsis*	Function	Refs
AmMYB308	*Antirrhinum majus*	AtMYB4	Repressor	[36]
AmMYB330	*A. majus*	AtMYB4	Repressor	[36]
AtMYB4	*Arabidopsis thaliana*		Repressor	[33]
AtMYB32	*A. thaliana*		Repressor	[37]
EgMYB1	*Eucalyptus gunnii*		Repressor	[4]
ZmMYB31	*Zea mays*		Repressor	[38]
ZmMYB42	*Z. mays*		Repressor	[35]
PttMYB21a	*Populus tremula* × *tremuloides*	AtMYB52	Repressor	[39]
VvMYB5a	*Vitis vinifera*		Repressor	[40]
BREVIPEDICELLUS (*BP*)	*A. thaliana*		Repressor	[34]
AtMYB46	*A. thaliana*		Activator	[14]
AtMYB83	*A. thaliana*		Activator	[13]
AtMYB58/63	*A. thaliana*		Activator	[3]
AtMYB85	*Arabidopsis*		Activator	[15]
AtMYB61	*A. thaliana*		Activator	[16]
PtMYB1	*Pinus taeda*	AtMYB85	Activator	[2]
PtMYB4	*P. taeda*	AtMYB46	Activator	[9]
PtMYB8	*P. taeda*	AtMYB46	Activator	[8]
EgMYB2	*E. gunnii*	AtMYB46	Activator	[11]
PtrMYB3	*Populus trichocarpa*	AtMYB46	Activator	[12]
PtrMYB20	*P. trichocarpa*	AtMYB46	Activator	[12]
AtNST1/NST3	*A. thaliana*		Activator	[23]
AtNST2	*A. thaliana*		Activator	[25]
AtMYB26	*A. thaliana*		Activator	[24]
NtMYBBJS1	*Nicotiana tabacum*		Activator	[20]
NtLIM	*N. tabacum*		Activator	[22]

Other phenylpropanoid pathway transcription factors also affect lignin biosynthesis. The *PAP1 MYB* gene was first characterized as a regulator of anthocyanin biosynthesis, but it also causes weak overaccumulation of lignin when overexpressed, because it upregulates genes shared by the anthocyanin and lignin pathways [19]. NtMYBBJS1 is a methyl jasmonate-dependent MYB transcription factor from tobacco (*Nicotiana tabacum*) that can upregulate *PAL* and *4CL* expression in tobacco BY-2 cell cultures. It is so far unclear whether this also occurs at the whole-plant level. [20].

MYBs are not the only transcription factors that can regulate the lignin pathway. NtLIM from tobacco regulates expression of *PAL*, *4CL* and *CAD*, and a reduction in lignin content of approximately 30% was observed when NtLIM was expressed in tobacco in the antisense orientation [21,22]. Three NAC transcription factors, NST1, NST2 and NST3, were shown to regulate the entire secondary cell wall program in *Arabidopsis* (Figure 2) [15]. The MYB master switches AtMYB46 and AtMYB83 are direct downstream targets of NST transcription factors. Not surprisingly, overexpression of NSTs causes ectopic lignification [23]. The NST homologs VND6 and VND7, which are involved in xylem vessel formation, were able to upregulate AtMYB46 directly, suggesting they also regulate the lignin pathway [13,24]. The identification of NST and its close homologs provided the first evidence indicating that the entire program of secondary cell wall formation is coordinately regulated. In *Arabidopsis*, NST1 and NST3 redundantly regulate secondary wall synthesis in the stem, whereas NST1 and NST2 function redundantly to promote secondary wall formation in anthers [24,25]. However, the model legume *Medicago truncatula* appears to have a single *NST* gene, and Mt*nst1* loss-of-function mutants show secondary wall biosynthesis defects in both stem and anthers [26].

Differences between the MYB46/MYB83 and NST master switches

Although pairs of *NST* genes and MYB46/MYB83 regulate the entire secondary wall biosynthesis program, and NSTs directly activate MYB46 and its close homologs, MYB46 appears to have the more direct and important role in the control of secondary wall formation. For example, promoter–GUS analysis indicates that the expression patterns of the two groups of transcription factor are not identical. In developing interfascicular fiber cells in rapidly elongating internodes that do not yet have secondary wall thickening, the *AtNST3* promoter shows activity, whereas there is little expression of *AtMYB46* [14,23]. This suggests that NSTs are not able to activate secondary wall thickening without expression of *MYB46*. Furthermore, plants with double knockouts of *AtNST1* and *AtNST3* are still viable, whereas plants with double knockout of *AtMYB46* and *AtMYB83* cannot grow further after developing the first pair of true leaves [13].

There might also be differences in the abilities of the MYB and NST switches to regulate lignin repressors (see below). MYB46 strongly activates the transcription of the repressors *MYB7* and *MYB32,* whereas NSTs do not [27]. However, this evidence comes from transient expression studies in which the transcription factors were expressed under control of the constitutive 35S promoter; therefore,

the regulation of lignin repressors by MYb46 *in vivo* is still to be resolved (Figure 2).

The role of lignin activators in reproductive tissues

Recently, it was shown that NST1 and NST3 regulate pod shattering by promoting secondary wall formation in siliques of *Arabidopsis* [28]. The spatial control of lignification in siliques is crucial for their development and dehiscence. Misexpression of the key transcriptional regulators of silique organ identity, such as the bHLH transcription factor ALCATRAZ (ALC) and MADS box gene FRUITFULL (FUL), results in developmental defects in organ identity and ectopic lignification in seed pods of *Arabidopsis* [29,30]. The fact that silique organ identity genes express normally in the *nst1 / nst3* double mutant indicates that NST1 and NST3 act after the establishment of silique tissue identity [28] (Figure 2). The different *NST* genes are only partially redundant in regulating lignification in siliques. Knocking out *nst1* in the *ful* mutant completely suppressed ectopic lignification. However, the lignification phenotype of the *ful:nst3* double mutant was the same as that of the *ful* mutant [28]. These results could be the result of the different expression patterns of *nst1* and *nst3*. *NST1* is expressed in both the valve margin and endocarp cells, whereas *NST3* is only expressed in the valve margin. How NST1-dependent lignification in siliques is activated by organ identity regulators is still a puzzle. Moreover, different monolignol biosynthesis genes can be involved in lignification in siliques compared with other organs. For example, *AtCAD1* is not expressed in interfascicular fibers of inflorescence stems, but is expressed in *Arabidopsis* siliques [31].

Arabidopsis AtMYB26 regulates the entire program of secondary wall biosynthesis in the endothecium of the anther [32], and its overexpression in tobacco caused ectopic lignification [32]. However, the regulation of secondary wall thickening by AtMYB26 is probably via NST1 and NST2 (Figure 2). Double knockouts of *AtNST1* and *AtNST2* had similar anther phenotypes to the *myb26* mutant; moreover, the expression of *NST1* and *NST2* appears to be regulated by AtMYB26 [25,32]. Analysis of the promoter regions of *NST1* and *NST2* also suggests that these contain MYB-binding sites [32]. However, AtMYB26 does not appear to regulate secondary wall thickening in inflorescence stems; whether there is a further transcription factor regulating *NST1* and *NST3* in inflorescence stems remains unknown.

Transcriptional repressors regulate monolignol biosynthesis

Several transcription factors have now been characterized as repressors of lignin biosynthesis [33–36]. In an early report, AmMYB308 and AmMYB330 from *Antirrhinum majus* downregulated *4CL*, *C4H* and *CAD* when overexpressed in tobacco [36]. AtMYB4, which is induced in response to UV-B in *Arabidopsis*, repressed the same genes when overexpressed in tobacco (Table 1) [33]. However, the CAD transcript level was not reduced in *Arabidopsis AtMYB4*-overexpressing lines, suggesting differential mechanisms for regulation of *CAD* genes in *Arabidopsis* and tobacco. Overexpression of both AmMYB308/330 and AtMYB4 in tobacco and *Arabidopsis* affected plant growth and development. Another MYB repressor from *Arabidopsis*, AtMYB32, appears to control the lignin pathway negatively through other targets; and the *Arabidopsis myb32* mutant shows slightly increased expression of *COMT* [37]. More recently, *Eucalyptus gunnii* EgMYB1 has also been proposed as a repressor of the monolignol pathway. It not only binds to the promoter of *EgCCR*, but also acts as negative regulator of *EgCCR* and *EgCAD in vivo* (Table 1) [4]. In maize (*Zea mays*), ZmMYB31 and ZmMYB42 also act as repressors of lignin biosynthesis (Table 1). Many genes in the monolignol pathway, including hydroxycinnamoyl CoA shikimate hydroxycinnamoyl transferase *(HCT)*, *F5H*, *C4H* and *4CL*, are downregulated in ZmMYB42-overexpressing *Arabidopsis*, which contains a lignin polymer with a decreased S:G ratio through the enrichment of H and G subunits and the depletion of S subunits [35,38].

All the repressors outlined above belong to subgroup 4 of the R2R3-MYB transcription factor family. Lignin repressors belonging to other MYB subgroups have also been reported. *Populus* PttMYB21a, which is a homolog of *Arabidopsis* AtMYB52, negatively regulates the expression of *CCoAOMT* when expressed in hybrid aspen plants in the antisense orientation, and acid soluble lignin levels were higher in the transgenic lines compared with wild-type plants (Table 1) [39]. Grapevine (*Vitis vinifera*) VvMYB5a, which regulates anthocyanin and proanthocyanidin biosynthesis in grapevine, also impacts lignin biosynthesis. When it is overexpressed in tobacco, two *CCoAOMT* genes are downregulated. Furthermore, lignified fibers were partially missing in anther cells, resulting in delayed dehiscence [40].

Recently, MYB75 (also known as PAP1), a positive regulator of anthocyanin biosynthesis in *Arabidopsis*, was reported to function as a repressor of the entire secondary cell wall program. A loss-of-function mutation in MYB75 (*myb 75-1*) resulted in increased cell wall thickness in xylary and interfascicular fibers within the inflorescence stem, associated with upregulation of genes involved in cellulose, xylan and lignin biosynthesis [41]. MYB75 is the first transcription factor reported so far that functions as a repressor of the entire secondary cell wall program, although it does not appear to have repressor activity in an *Arabidopsis* protoplast transfection system that measures promoter activities [41]. It is currently unclear how MYB75 represses secondary cell wall biosynthesis and whether MYB75 is regulated by the secondary cell wall biosynthesis master switches NST1/NST2/NST3 and MYB46/MYB83. The apparent discrepancy between the previously reported increase in lignin levels in *PAP1*-overexpressing *Arabidopsis* could be a result of the different methods used for lignin analysis in the two studies, one gravimetric [41], and one relying on chemical degradation and, therefore, affected by lignin composition and linkage types [19].

BREVIPEDICELLUS (*BP*), one of the seven *knotted1*-like homeobox (*KNOX*) genes in *Arabidopsis*, which have important roles in plant meristems by regulating cell division and differentiation, is also involved in lignin pathway regulation. Lignin deposition is increased in *bp*

mutants and decreased in *BP* overexpression lines. The promoters of *COMT* and *CCoAOMT* can be bound by BP. Transcripts of many lignin genes are slightly, but significantly, increased in the *bp* mutant [34].

Similar to the lignin transcriptional activators described above, most transcriptional repressors can physically bind to AC-rich elements in the promoter regions of lignin biosynthesis genes [2,4,22,34]. However, lignin transcriptional repressors only specifically downregulate some of the lignin biosynthesis genes, although most have AC-rich elements in their promoter regions. The mechanisms underlying the selective binding of lignin transcriptional repressors to promoters of specific lignin biosynthesis genes are still unclear.

The expression of lignin repressors is controlled both developmentally and environmentally

The fine spatial and temporal control of lignin deposition relies on the antagonistic action of both repressors and activators to ensure the precise level of lignin accumulation in specific cells, tissues or organs. Unlike lignin biosynthesis genes and activators, which are preferentially expressed in actively lignifying tissues during development, many lignin repressors are preferentially expressed in non- or poorly lignifying tissues. For instance, AtMYB32 is more highly expressed in flowers than in highly lignifying tissues, such as stem, and this accounts for the lower levels of lignin observed in floral tissues than in stems. The *KNOX* gene family member *BP* is thought to maintain cells in an indeterminate state [42], and it is important to prevent premature lignin deposition, which is considered a signature for irreversible cell differentiation.

The activation of lignin deposition in plant defense can occur through the repression of lignin repressors rather than the induction of activators. It is well known that lignin is induced in response to environmental cues, such as wounding, mechanical stress and plant pathogens, and some monolignol pathway genes are specifically induced by stress [43,44]. The *Arabidopsis* lignin repressor AtMYB4 was shown to be downregulated both in wounded leaves and in systemically non-wounded leaves from the same rosette. It is reasonable to speculate that the decrease in AtMYB4 levels upregulates the monolignol pathway so that lignin can be produced in wounded tissues as a defense strategy.

Modification of transcription regulators

Despite the crucial role of transcriptional regulators in monolignol biosynthesis, the potential for modification of these proteins or their possible binding partners has received surprisingly little attention. Recently, evidence from *P. taeda* has shown that the lignin activators PtMYB1 and PtMYB4 are phosphorylated by a MAP kinase, PtMAPK6. This post-translational modification does not alter DNA binding, but promotes transcriptional activation in yeast [45]. The MAPK phosphorylation site is also present in other plant lignin pathway MYBs. However, the biological importance of such phosphorylation in monolignol biosynthesis is currently unknown.

Recently, it has been shown that ectopic lignin deposition in response to biotic stress is modulated by poly (ADP-ribosyl)ation. In *Arabidopsis*, exogenous application of an inhibitor of poly(ADP-Rib) polymerase can block lignin deposition, which is otherwise induced as one of the innate immune responses by microbe-associated molecular patterns, such as bacterial flagellin [46]. Given that poly(ADP-ribosyl)ation is a post-translational protein modification, it is reasonable to speculate that such modification occurs on one or more transcriptional regulators of the monolignol biosynthesis pathway. Some lignin repressors are induced in response to wounding [33], and it is therefore worth investigating the possibility that these undergo poly(ADP-ribosyl)ation in response to biotic stress.

The UV-B-induced AtMYB4 was shown to regulate its own gene expression negatively, with the AC elements present in the *AtMYB4* promoter serving as its own binding site [47]. However, the biological importance of such feedback inhibition *in planta* is currently unknown.

The expression of lignin pathway transcriptional regulators is affected by plant hormones

Increasing evidence indicates links between plant hormone actions and changes in lignification. In fact, most major plant hormones affect the lignin biosynthesis pathway. *AtMYB32* is greatly upregulated by indole 3-acetic acid (IAA) [37] (Figure 2), and the *KNOX* gene family member *BP* negatively regulates lignin biosynthesis and its expression is repressed by auxin [34] (Figure 2). The expression of the lignin repressor PtMYB21a from hybrid aspen is upregulated by both IAA and GA but downregulated by cytokinin [39]. Mutants in primary cellulose synthase (*cesa*1 and *cesa*3), V-ATPase and glycerophosphoryl diester phosphodiesterase-like protein show ectopic lignification along with increased level of JA-related genes or JA precursors [48–50]. In the case of the V-ATPase mutant, AtMBY61 is misexpressed and suppression of AtMYB61 can restore the phenotype. It is therefore possible that JA affects the lignin biosynthesis pathway at the transcriptional level through AtMYB61. A mutant in a chitinase-like gene shows ectopic lignification and overproduces ethylene [51]. Mutants in two leucine-rich-repeat receptor-like kinases, which can be rescued by the ethylene inhibitors α-aminoisobutyric acid, aminoox- acetic acid and 1-methylcyclopropene, also show ectopic deposition of lignin [52]. Katanin-like microtubule-severing protein (*ktn1*) mutants show increased lignin along with overexpression of GA biosynthesis genes [53,54]. Despite many studies linking plant hormones to lignification, the key regulatory nodes in the lignin pathway sensing plant hormone changes are still to be identified.

The lignin biosynthesis pathway crosstalks with other physiological processes

Many lignin pathway genes are under the control of light, the circadian clock and sugar levels. For example, *C4H*, *COMT*, *CCoAOMT* and *CCR1* exhibit circadian fluctuations in transcript abundance [55,56], and it has been suggested that metabolizable sugars positively influence the abundance of lignin [56]. In fact, circadian changes in transcript abundance of lignin biosynthesis genes could be a secondary effect of changes in plant carbon metabolism. Generally, plants synthesize starch during the daytime

and then turn it over to sucrose at night. There could be a mechanism to sense sugar levels for controlling lignin biosynthesis so that plants can optimize carbon resource availability because both starch and lignin biosynthesis consume large amounts of carbon. Consistent with this idea, the *sex1 Arabidopsis* mutant, which has a defect in starch degradation, expresses seven of eight tested lignin biosynthesis genes at a lower level compared with wild-type control plants. This effect could be the result of reduced levels of carbon released from the starch pool [56]. Another example of carbon-level modulating lignin biosynthesis is the observation that lignin biosynthesis genes are upregulated by the addition of sucrose in dark-grown *Arabidopsis* [56]. It is now important to identify lignin transcriptional regulators that sense carbon metabolism to determine the mechanisms underlying the control of lignin biosynthesis by light, circadian clock and sugar levels.

Conclusions

During the past decade, a big step forward towards a better understanding of the transcriptional regulation of lignin biosynthesis has been made. Many transcriptional regulators from different gene families and different species have been identified and characterized. It has been widely accepted that the AC-rich *cis*-elements are involved in the regulation of lignin gene expression. Recent studies on secondary cell wall biosynthesis in *Arabidopsis* have revealed that lignin production is coordinately regulated along with the two other major components of the secondary cell wall, cellulose and xylan, by the same group of NAC transcription factors [23,24]. The identification of so-called 'master switches', which activate the entire secondary cell wall formation program, has provided a good starting point for a genome-wide search for downstream transcriptional regulators (Figure 2) [26,27].

Despite this progress, three major questions still remain. (i) The mechanisms underlying the response of lignin regulators to plant hormones, sugars and stress are unclear. The complex synthesis, transport and signaling events of plant hormones, sugars and stress-related molecules make it difficult to distinguish between primary versus secondary effects on lignin biosynthesis. Identification of the lignin regulators that are also affected by plant hormones, sugars and/or stress would be a breakthrough to connect the lignin biosynthesis pathway with other biological processes. (ii) The biological importance of the modification of some lignin transcriptional activators is largely unknown. Whether poly(ADP-ribosyl)ation has effects on the transcriptional regulation of lignin biosynthesis, or whether the MAPK phosphorylation of lignin activators controls lignin biosynthesis *in vivo* are interesting and important puzzles that remain to be solved. (iii) The fact that some lignin biosynthesis genes can be regulated by both activators and repressors suggests that regulation of lignin biosynthesis involves a yet unknown fine-tuning mechanism, providing extra flexibility of transcriptional control by activators and repressors on common targets. Addressing these questions will require a better understanding of the crosstalk between the lignin and other plant physiological pathways.

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