

MYB transcription factors in *Arabidopsis*

Christian Dubos¹, Ralf Stracke², Erich Grotewold³, Bernd Weisshaar², Cathie Martin⁴ and Loïc Lepiniec¹

¹ Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech, 78026 Versailles Cedex, France

² Bielefeld University, Department of Biology, Genome Research, 33594 Bielefeld, Germany

³ Department of Plant Cellular and Molecular Biology and Plant Biotechnology Center, Ohio State University, Columbus, OH 43210, USA

⁴ John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

The MYB family of proteins is large, functionally diverse and represented in all eukaryotes. Most MYB proteins function as transcription factors with varying numbers of MYB domain repeats conferring their ability to bind DNA. In plants, the MYB family has selectively expanded, particularly through the large family of R2R3-MYB. Members of this family function in a variety of plant-specific processes, as evidenced by their extensive functional characterization in *Arabidopsis* (*Arabidopsis thaliana*). MYB proteins are key factors in regulatory networks controlling development, metabolism and responses to biotic and abiotic stresses. The elucidation of MYB protein function and regulation that is possible in *Arabidopsis* will provide the foundation for predicting the contributions of MYB proteins to the biology of plants in general.

MYB transcription factors in plants

More than 20 years ago, the first gene encoding a transcription factor in plants was identified; the *COLORED1* (*C1*) locus was found to encode a MYB domain protein required for the synthesis of anthocyanins in the aleurone of maize (*Zea mays*) kernels [1]. A decade ago, the *Arabidopsis* genome sequence was published, which provided the first comprehensive description and classification of plant MYB genes [2]. Since then, a tremendous amount of data has accumulated on the roles of MYB transcription factors in plants [3]. The functions of MYB proteins have been investigated in numerous plant species such as *Arabidopsis*, maize, rice (*Oryza sativa*), petunia (*Petunia hybrida*), snapdragon (*Antirrhinum majus*), grapevine (*Vitis vinifera* L.), poplar (*Populus tremuloides*) and apple (*Malus domestica*), using both genetic and molecular analyses. Concomitantly, new insights have been obtained into the mechanisms that control MYB protein activities, and gene expression profiles and several target genes have been determined. However, few DNA-binding sites have been characterized functionally; these can be found in databases (e.g. <http://arabidopsis.med.ohio-state.edu/> or <http://www.dna.affrc.go.jp/PLACE/>). The increasing availability of plant genome sequences has allowed comparisons and a better understanding of the evolution of this large

family of transcription factors. Here, we review recent advances in understanding the R2R3-MYB family in *Arabidopsis* (Figure 1) with particular emphasis on their biological roles (Table S1 in online supplementary material), because more than half of these have been identified within the past three years (Figure S1 in online supplementary material). Our current knowledge of the mechanisms by which *Arabidopsis* R2R3-MYB activities are regulated is also summarized.

Structure and evolution

MYB proteins are characterized by a highly conserved DNA-binding domain: the MYB domain. This domain generally consists of up to four imperfect amino acid sequence repeats (R) of about 52 amino acids, each forming three α -helices. The second and third helices of each repeat build a helix–turn–helix (HTH) structure with three regularly spaced tryptophan (or hydrophobic) residues, forming a hydrophobic core in the 3D HTH structure [4]. The third helix of each repeat is the “recognition helix” that makes direct contact with DNA and intercalates in the major groove ([5] and references therein). During DNA contact, two MYB repeats are closely packed in the major groove, so that the two recognition helices bind cooperatively to the specific DNA sequence motif.

MYB proteins can be divided into different classes depending on the number of adjacent repeats (one, two, three or four; Figure 1). The three repeats of the prototypic MYB protein c-Myb are referred to as R1, R2 and R3, and repeats from other MYB proteins are named according to their similarity to R1, R2 or R3 of c-Myb. All four classes are found in plants, representing the taxon with the highest diversity of MYB proteins. The smallest class is the 4R-MYB group, whose members contain four R1/R2-like repeats. A single 4R-MYB protein is encoded in several plant genomes. Little more is known of these proteins in plants.

The second class contains R1R2R3-type MYB (3R-MYB) proteins, typically encoded by five genes in higher plant genomes. Genes encoding 3R-MYB proteins have been found in most eukaryotic genomes, so they represent a conserved gene class with roles, albeit divergent, in cell cycle control [6,7].

The third heterogeneous class comprises proteins with a single or a partial MYB repeat, collectively designated

Corresponding authors: Dubos, C. (christian.dubos@versailles.inra.fr); Lepiniec, L. (Loic.Lepiniec@versailles.inra.fr).

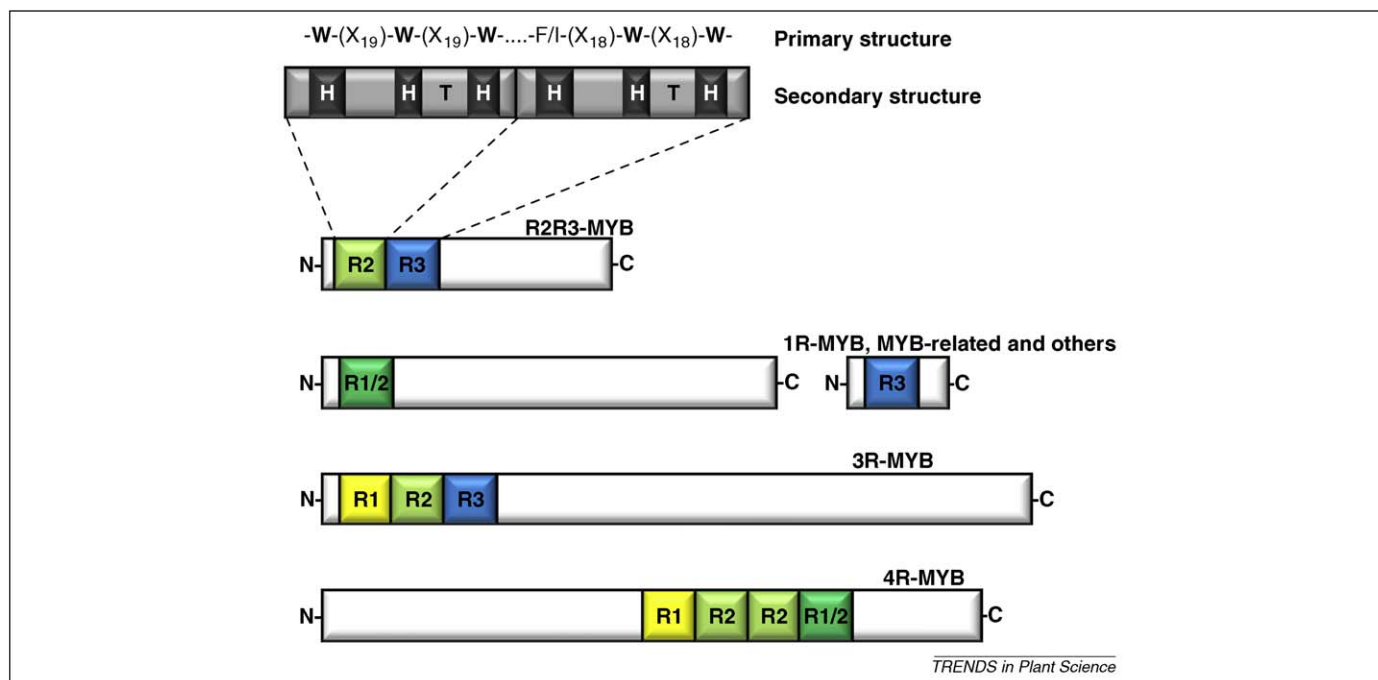


Figure 1. Plant MYB transcription factor classes. Illustration showing different plant MYB protein classes, depending on the number of adjacent MYB repeats (R). The primary and secondary structures of a typical R2R3-MYB are indicated. H, helix; T, turn; W, tryptophan; X, amino acid (X).

“MYB-related” that fall into several subclasses [8] (Figure 1). Those of the R3-type, which includes *TRIPTYCHON* (*TRY*), *CAPRICE* (*CPC*) and *MYBL2*, are likely to have evolved from *R2R3-MYB* genes and are involved in the control of cellular morphogenesis [9,10] and in secondary metabolism control [11,12]. Those MYB-related genes of the evolutionarily older R1/R2-type, including *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*), encode core components of the central circadian oscillator [13]. Last, those of the *GARP* family [14], including *KANADI* [15], *GOLDEN2-LIKE* [16] and *PHR1* [17], encode proteins involved in organ morphogenesis, chloroplast development and the responses to phosphate starvation, respectively.

Most plant *MYB* genes encode proteins of the R2R3-MYB class, which are thought to have evolved from an *R1R2R3-MYB* gene ancestor, by the loss of the sequences encoding the R1 repeat and subsequent expansion of the gene family [8]. However, the evolution of 3R-MYB genes from *R2R3-MYB* genes by the gain of the sequences encoding the R1 repeat through an ancient intragenic duplication has also been proposed [18]. R2R3-MYB transcription factors have a modular structure, with an N terminal DNA-binding domain (the MYB domain) and an activation or repression domain usually located at the C terminus. In contrast to the highly conserved MYB domain, the other regions of R2R3-MYB proteins are highly variable. Based on the conservation of the DNA-binding domain and of amino acid motifs in the C terminal domains, R2R3-MYB proteins have been divided into subgroups [2] (Figure 2). Most of these subgroups, defined first for the proteins of *Arabidopsis*, are also present, and are sometimes expanded, in other angiosperms [2,19]. Comparative phylogenetic studies have identified new R2R3-MYB subgroups in other plant species for which there are

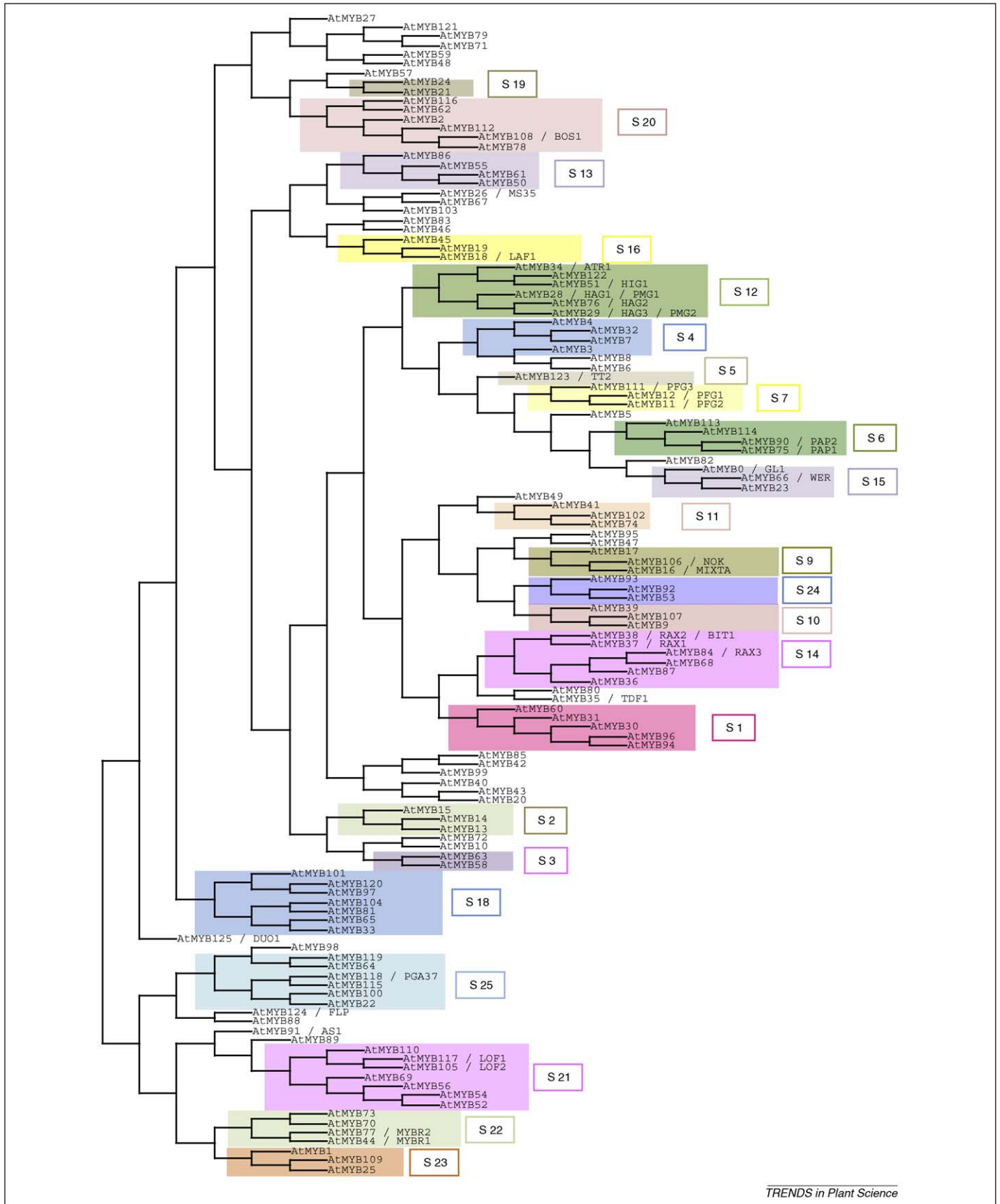
no representatives in *A. thaliana* (e.g. in poplar and grapevine), suggesting that these proteins might have specialized roles which were either lost in *Arabidopsis* or were acquired after divergence from the last common ancestor [20]. The expansion of the *R2R3-MYB* gene family in plants (Table 1), which typically contain more than 100 members [2,20–24], fits well with the observation that many (if not all) R2R3-MYB transcription factors play central roles in plant-specific processes [25].

Diversity of functions

Numerous R2R3-MYB proteins have been characterized by genetic approaches and found to be involved in the control of plant-specific processes including (i) primary and secondary metabolism, (ii) cell fate and identity, (iii) developmental processes and (iv) responses to biotic and abiotic stresses (Table S1 in online supplementary material).

Regulation of primary and secondary metabolism in *Arabidopsis*

Several R2R3-MYBs are involved in the regulation of flavonoid biosynthesis (Figure 3). For instance, *AtMYB11/PFG1*, *AtMYB12/PFG1* and *AtMYB111/PFG3* (subgroup 7, Table S1 in online supplementary material) control flavonol biosynthesis in all tissues [26], *AtMYB75/PAP1*, *AtMYB90/PAP2*, *AtMYB113* and *AtMYB114* (subgroup 6) control anthocyanin biosynthesis in vegetative tissues [27] and *AtMYB123/TT2* (subgroup 2) control the biosynthesis of proanthocyanidins (PAs) in the seed coat of *Arabidopsis* [28]. In addition, *AtMYB5* was recently proposed to be partially redundant with *AtMYB123* in regulating tannin biosynthesis [29]. These functions are broadly conserved for the MYB proteins of the same subgroup in different angiosperms and, although members of subgroup 7 regulate the production of somewhat different



TRENDS in Plant Science

Figure 2. Schematic representation of the relationships between the different R2R3-MYB subgroups. The tree was inferred using the neighbor-joining method and 1000 bootstraps with putative amino acid full length MYB sequences with Clustal X2 software. The subgroups were designated as previously reported [2]. In this later report, *AtMYB123* (*TT2*) was grouped with a maize gene “C1” to provide the subgroup 5.

Table 1. Numbers of the members in the four different MYB classes

MYB protein classes	Eudicot			Monocot	Moss
	<i>A. thaliana</i>	<i>Populus trichocarpa</i>	<i>V. vinifera</i>	<i>O. sativa</i>	<i>P. patens</i>
R2R3-MYB	126	192	108	109	61
1R-MYB, MYB-related	64	n.d.	n.d.	70	31
3R-MYB	5	5	5	5	2
4R-MYB	1	–	1	1	1
Refs	[2,22]	[20]	[24]	[22]	[*]

Abbreviations: n.d., not determined, *, the Moss Transcription Factor Database (<http://plantfdb.cbi.pku.edu.cn/index.php>).

phenylpropanoids in different species, they regulate many target genes in common. Within a subgroup, paralogs can control the same metabolic pathway in different cell types as a result of differences in expression patterns [26–28]. *AtMYB3*, *AtMYB4*, *AtMYB7* and *AtMYB32* (subgroup 4) encode transcriptional repressors [11,30,31]. *AtMYB4* controls sinapate ester biosynthesis in a UV-dependent manner [30], whereas *AtMYB32* regulates pollen wall composition [31]. Other MYB proteins are involved in the control of cell wall biosynthesis. *AtMYB58*, *AtMYB63* (subgroup 3) and *AtMYB85* activate lignin biosynthesis in fibers and/or vessels [32,33], whereas *AtMYB68* negatively regulates lignin deposition in roots [34]. *AtMYB46* is a positive regulator of lignin biosynthesis in fibers and vessels and also regulates cellulose and xylan deposition [35]. *AtMYB26/MS35* controls secondary wall deposition in anthers [36]. *AtMYB52*, *AtMYB54* and *AtMYB69* (subgroup 21) and *AtMYB103* are positive regulators dedicated to cell wall thickening in fiber cells. *AtMYB52*, *AtMYB54* and *AtMYB69* are proposed to regulate lignin, xylan and cellulose biosynthesis, and *AtMYB103*, cellulose biosynthesis [33]. *AtMYB61* (subgroup 13) plays a pleiotropic role, influencing lignin deposition [37], mucilage production [38] and stomatal aperture [39], suggesting that it might act upstream of the different pathways perhaps by regulating carbon allocation. The R2R3-MYB proteins of subgroup 12 regulate glucosinolate biosynthesis and *AtMYB28/HAG1/PMG1*, *AtMYB29/HAG3/PMG2* and *AtMYB76/HAG2* regulate the biosynthesis of aliphatic glucosinolates in aerial issues [40,41]. However, *AtMYB34/ATR1*, *AtMYB51/HIG1* and *AtMYB122* regulate the production of indolic glucosinolates in roots and late stage rosette leaves [42].

Regulation of cell fate and identity in *Arabidopsis*

The determination of epidermal cell type involves several R2R3-MYB proteins encoded by *AtMYB0/GL1*, *AtMYB23* and *AtMYB66/WER* (subgroup 15). *AtMYB0* and *AtMYB23* control trichome initiation in shoots, whereas *AtMYB66* controls root hair patterning. In roots, *AtMYB23* is positively regulated by *AtMYB66* and participates in a positive feedback loop to reinforce the cell fate establishment process [43]. *AtMYB23* regulates trichome extension and branching in combination with *AtMYB5* [44,45]. *AtMYB5* also regulates outer seed coat differentiation [29,45]. Subgroup 9 contains MIXTA-like transcription factors: *AtMYB106/NOK*, a negative regulator of trichome branching [46], *AtMYB16/MIXTA*, proposed to control the shape of petal epidermal cells [47], and *AtMYB17*, a putative regulator of early inflorescence development and seed germination [48].

Stomatal differentiation and patterning are strictly coordinated in time and space. Two closely related

R2R3-MYBs, *AtMYB88* and *AtMYB124/FLP*, act in this process by restricting divisions late in the stomatal cell lineage and inducing terminal differentiation [49], by regulating genes involved directly in cell cycle progression [50]. *AtMYB98* regulates synergid cell differentiation during female gametophyte development, pollen tube guidance and the formation of the filiform apparatus [51].

Regulation of plant development by R2R3-MYB proteins in *Arabidopsis*

Several R2R3-MYB genes control anther development and/or functionality including *AtMYB21*, *AtMYB24* (subgroup 19), *AtMYB57*, *AtMYB108/BOS1*, *AtMYB35/TDF1*, *AtMYB80* (formerly *AtMYB103*) and *AtMYB99* [52,53]. *AtMYB24* and *AtMYB108* are redundant, acting downstream of *AtMYB21* [53]. *AtMYB80* regulates exine formation and acts downstream of *AtMYB35* [54]. *AtMYB125/DUO1* is a pollen-specific factor controlling male germ cell division and differentiation [55]. *AtMYB33* and *AtMYB65* (subgroup 18) redundantly facilitate both anther and pollen development [56].

AtMYB37/RAX1, *AtMYB38/RAX2/BIT1* and *AtMYB84/RAX3* (subgroup 14) are partially redundant regulators of axillary meristem formation. *AtMYB37* acts early during the vegetative phase, whereas *AtMYB38* affects accessory side shoot formation during inflorescence development, both having a greater impact than *AtMYB84* [57,58]. *AtMYB105/LOF2* and *AtMYB117/LOF1* (subgroup 21) control lateral organ separation and axillary meristem formation upstream of *AtMYB37* [59]. *AtMYB91/AS1* regulates shoot morphogenesis and leaf patterning through its competitive actions with KNOX proteins [60], although in other species related proteins have broader functions, determining the dorso-ventral polarity of leaves and so influencing cell density. In seedlings, *AtMYB38* and *AtMYB18/LAF1* (subgroup 16) regulate hypocotyl elongation in response to blue [61] and far-red light [62], respectively. *AtMYB115* and *AtMYB118/PGA37* (subgroup 25) have been proposed to play roles in embryogenesis [63]. *AtMYB59* regulates root development through the control of cell cycle progression at the root tips [64] and *AtMYB77* (subgroup 22) regulates lateral root formation by modulating the expression of auxin-inducible genes [65]. *AtMYB68* (subgroup 14) is a root growth-specific regulator, impacting overall plant development under unfavorable conditions (e.g. high temperature) [34].

Responses of *Arabidopsis* to biotic and abiotic stresses

As autotrophic sessile organisms, plants have developed various strategies to respond to unfavorable environmental conditions. R2R3-MYB proteins such as *AtMYB30*, *AtMYB60* and *AtMYB96* (subgroup 1) are involved in these

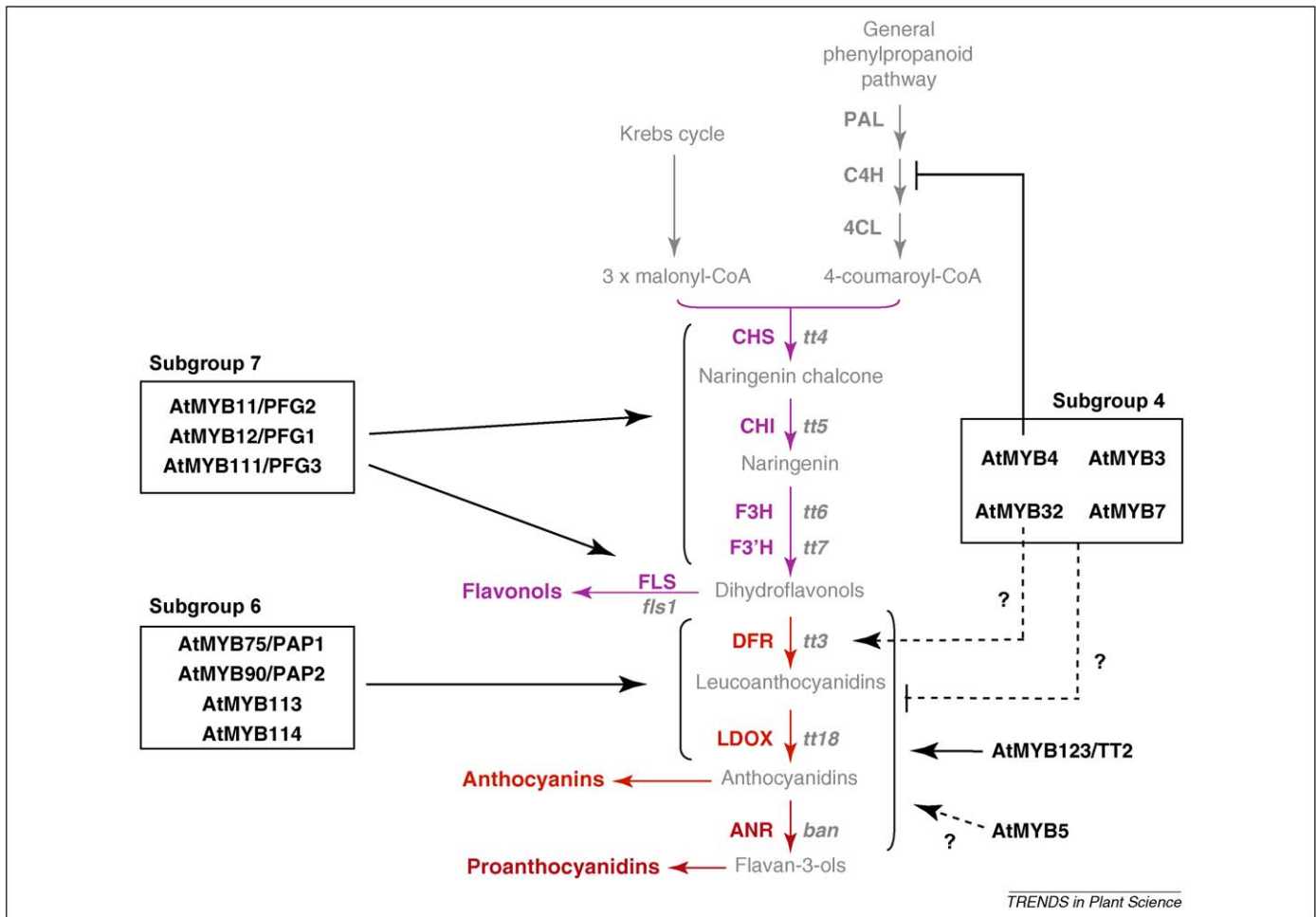


Figure 3. R2R3-MYB regulation of the flavonoid biosynthetic pathway (adapted from [11,28]). Flavonoid biosynthesis is initiated by the condensation of three molecules of malonyl-CoA (from the Krebs cycle) with one molecule of 4-coumaroyl-CoA. Early biosynthetic genes (EBGs: *CHS*, *CHI*, *F3H*, *F3'H* and *FLS1*) catalyze the flavonol biosynthesis, whereas late biosynthetic genes (LBGs: *DFR*, *LDOX* and *ANR*) lead to both anthocyanin and PA biosynthesis. EBGs are positively regulated by subgroup 7 R2R3-MYBs, whereas LBGs are activated by subgroup 6 R2R3-MYBs or *AtMYB123* when leading to the biosynthesis of anthocyanins and PAs, respectively. When ectopically expressed in PA-accumulating cells, all subgroup 4 R2R3-MYB members were able to inhibit PA biosynthesis, suggesting that these proteins have the ability to inhibit LBG expression. *AtMYB5* was proposed to act redundantly with *AtMYB123*. Abbreviations: PAL, phenylalanine ammonium lyase; C4H, cinnamate-4-hydroxylase; 4CL, 4-coumaroyl-CoA synthase; CHI, chalcone isomerase; F3H, flavonol 3-hydroxylase; F3'H, flavonol 3'-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol-4-reductase; LDOX, leucoanthocyanidin dioxygenase; ANR, anthocyanidin reductase. Enzymes are indicated in upper case letters and genetic loci in lower case italics. Dotted lines represent putative regulations.

responses. *AtMYB30* encodes an activator of the hypersensitive cell death program in response to pathogen attack, acting through the regulation of very-long-chain fatty acids synthesis. In seedlings, *AtMYB30* has also been shown to act in the brassinosteroid pathway controlling hypocotyl cell elongation [66,67]. *AtMYB60* and *AtMYB96* act through the ABA signaling cascade to regulate stomatal movement [68], and drought stress and disease resistance [69,70], respectively. *AtMYB44/AtMYBR1* (subgroup 22) regulates ABA-mediated stomatal closure in response to abiotic stresses and three other members of this subgroup (*AtMYB70*, *AtMYB73* and *AtMYB77/AtMYBR2*) are likely to be associated with stress responses [71]. *AtMYB13*, *AtMYB15* (subgroup 2), *AtMYB33* and *AtMYB101* (subgroup 18; [72]) are involved in ABA-mediated responses to environmental signals. *AtMYB15* is also involved in cold stress tolerance [73]. Three members of subgroup 20 are implicated in stress responses: *AtMYB2* controls the ABA induction of salt and dehydration responsive genes [74], *AtMYB62* is involved in the response to phosphate

starvation [75] and *AtMYB108* in both biotic and abiotic stress responses [76]. *AtMYB102/AtM4* and *AtMYB41* (subgroup 11) contribute to plant resistance against insects and probably affect dehydration after wounding [77] and osmotic stress responses [78,79], respectively. *AtMYB72* is a key regulator required in roots during early signaling steps of induced systemic resistance mediated by beneficial fungi and bacteria [80,81].

Regulation of MYB functions

MYB genes are targets to both microRNAs (miRNAs) and transacting, silencing RNAs (*ta*-siRNAs). For example, miR159 targets *AtMYB33*, *AtMYB35*, *AtMYB65* and *AtMYB101* [82], which are involved in anther/pollen development. The *Arabidopsis* MPSS Plus database (<http://mpss.udel.edu/at/>) currently lists miR828 as complementary to *AtMYB113* and *AtMYB82*, miR159c to *AtMYB81*, miR858 to *AtMYB12* and *AtMYB83* and miR159a to *AtMYB120*. *AtMYB13*, *AtMYB20* and *AtMYB111* have been confirmed as miR858 targets [83]. TAS4-siR81(-) is

predicted to target mRNAs for *AtMYB75*, *AtMYB90* and *AtMYB113* [84], which are involved in the control of anthocyanin biosynthesis [27]. Although the consequences of the knockouts of the two miR159s (miR159a and miR159b) have been reported to increase the accumulation of *AtMYB33* and *AtMYB65* mRNA [82], the *in vivo* roles of other miRNAs or the *TAS4* siRNAs remain unclear. Mutations affecting miRNA processing, such as *serrate* [85], do not induce transcript levels of these putative *MYB* targets (although transcript levels of *AtMYB40* and *AtMYB17* are elevated in *serrate* mutant seedlings), suggesting that they are not regulated by these miRNAs *in vivo*.

Post-translational modifications and protein–protein interactions can significantly impact the regulatory activity of *MYB* transcription factors. *MYB* proteins with two or more *MYB* repeats bind DNA *in vitro* as monomers; the tandem arrangement and cooperative interaction between the *MYB* repeats means they behave like “covalently linked” dimers when contacting DNA [86]. *MYB* proteins with just one *MYB* repeat, such as CCA1 and LHY [13], form heterodimers as well as homodimers. Presumably, dimerizations permit these proteins to recognize DNA with high affinity and specificity.

The transcriptional activity of some R2R3-*MYB* factors is dependent *in vivo* on protein–protein interactions. Best described is the cooperation between subgroup 15 R2R3-*MYB* factors [2] and basic helix–loop–helix (bHLH) proteins from subgroup IIIf [87]. The specificity of the interaction between *MYB* and the N terminal region of bHLH proteins has been mapped to a signature motif ((D/E)Lx₂(R/K)x₃Lx₆Lx₃R) in R3 repeat [88,89]. This motif is present in 14 R2R3-*MYB* and six R3-*MYB* proteins involved in the regulation of trichome patterning [90] or tannin biosynthesis in *Arabidopsis* [91]. The ability of the R3 proteins to interact with bHLH proteins is crucial to their negative regulatory functions because they compete for binding with the R2R3-*MYB* partners, but do not, themselves, bind DNA stably. Interactions with other proteins can modulate other R2R3-*MYB* activities, such as *AtMYB30* with BES1, *AtMYB77* with ARF7 and *AtMYB18* with both FHY1 and FHL (involved in brassinosteroid, auxin and light signaling, respectively) [62,65,66].

Phosphorylation is important in determining *MYB* protein activity. The tobacco (*Nicotiana tabacum*) 3R-*MYB* regulator NtMYB2 is positively regulated by phosphorylation by a cyclin-dependent kinase complex, which abolishes the negative regulatory activity of the C terminal region [92]. Similar regulatory mechanisms have been proposed for the control of the *Arabidopsis* *KNOLLE* syntaxin by *AtMYB3R1* and *AtMYB4* [7]. The transcriptional activity of the R2R3-*MYB* *Pinus taeda* PtMYB4 protein is positively regulated by the mitogen-activated protein kinase (MAPK) PtMAPK6, which phosphorylates a Ser in the C terminal activation domain, and similar phosphorylation might regulate other R2R3-*MYB* proteins such as *AtMYB46* in *Arabidopsis* [93]. Although phosphorylation often increases transcriptional activation functions, casein kinase 2 (CK2) holoenzyme stimulates the DNA-binding activity of the *Arabidopsis* CCA1 protein [94].

Redox control is thought to influence *MYB* protein activity because of the presence of a pair of conserved Cys residues, four residues apart, in the R2-*MYB* motif of most 3R- and R2R3-*MYB* proteins [95]. Under oxidizing conditions these two Cys residues in the maize P1 protein form an intramolecular S–S bond that significantly alters *MYB* domain structure, preventing DNA binding [96]. The similarity of P1 to *AtMYB11*, *AtMYB12* and *AtMYB111* suggests that redox control is at play in the regulation of these proteins in *Arabidopsis* [26,97]. A small subset of *Arabidopsis* R2R3-*MYB* proteins including *AtMYB2*, which controls salt and dehydration responsive genes [74], lacks the first Cys residue, making the formation of an intra-molecular S–S bond impossible. Nevertheless, the DNA-binding activity of *AtMYB2* is controlled by an alternative mechanism that involves cysteine *S*-nitrosylation [98]. Both oxidation and *S*-nitrosylation will negatively influence the DNA-binding activity of these transcription factors.

The activity of many transcriptional regulators is modulated by conjugation with ubiquitin or the small ubiquitin-related modifier (SUMO) [99,100]. The ubiquitination of transcription factors occurs at Lysine residues often at or close to the transcription activation domain (TAD), enhancing transcriptional activity before promoter clearance by targeting the ubiquitinated regulator for turnover via the ubiquitin–proteasome pathway [101,102]. In *Arabidopsis*, *AtMYB18*, for example, is ubiquitinated by COP1 [103], but the stability of *AtMYB18* can be increased by interaction with the bHLH factor HFR1 [104].

The modulation of transcription factor activity by sumoylation can occur by multiple mechanisms including subcellular localization, DNA-binding activity or decreasing the activity of a TAD, as described for animal c-Myb [105]. Similarly, the activity of PHR1, and thereby the response of *Arabidopsis* to phosphate deficiency, is controlled by the SUMO E3 ligase SIZ1 [106], suggesting sumoylation as a general mechanism for modulating the regulatory activity of *MYB* and other transcription factors [107].

MYB proteins in regulatory networks

MYB transcription factors have been identified as immediate targets of other regulators (87 instances currently documented in <http://arabidopsis.med.ohio-state.edu/>). One example is AGL15, a member of the MADS family involved in embryo development, which directly binds to 29 different *MYB* genes from a total of ~2000 target genes, although binding to DNA is not always synonymous with transcriptional regulation [108]. Genes directly regulated by *MYB* factors have started to emerge, and currently AtRegNet [109] harbors information on direct targets for 3R-*MYBs* (*MYB3R4* and *MYB3R1*) and five R2R3-*MYBs*, including *AtMYB123*, *AtMYB2*, *AtMYB66*, *AtMYB0* and *AtMYB33*, totaling 552 direct targets. Most of these correspond to direct targets of the *AtMYB0* regulator of trichome initiation, although only a small fraction (~20) of these *AtMYB0* targets are shared by its bHLH partner GL3 [110], suggesting that *AtMYB0* can control the expression of many genes independently of GL3, possibly in association with other bHLH factors. Among the *AtMYB0* targets

are numerous other transcription factors, as is the case for *AtMYB66*, which operates in the roots. Similar to *AtMYB0*, *AtMYB66* directly controls the *GL2* (homeodomain) and *CPC* (R3-MYB) genes [111]. By contrast, other MYB factors for which direct targets have been identified seem to target primarily non-transcription factor genes [7,74,91]. The picture that emerges is that MYB transcription factors function at multiple levels in hierarchical regulatory networks. Once more data are available, it will be interesting to establish how the control of specific target genes relates to the biological functions that MYB factors control.

Concluding remarks

The duplication of regulatory genes plays a key role in generating diversity. It is not surprising that the large family of plant-specific *R2R3-MYB* genes has contributed to the evolution of physiological or developmental processes specific to plants, especially those involved in responses to fluctuating biotic or abiotic environments. Analysis of new plant genomes suggests that some *MYB* genes have evolved to fulfill lineage-specific functions [112]. Within subgroups that are conserved between divergent species, primary protein structures and biological functions are correlated (e.g. phenylpropanoid metabolism regulation by *R2R3-MYB* subgroups 3, 4, 5, 6 and 7). Therefore, protein structure and expression patterns can help deduce new *MYB* functions in *Arabidopsis* as well as in other plants. The functions of *Arabidopsis* *MYB* proteins controlling anthocyanin and flavonol biosynthesis were predicted this way [26,28]. Similarly, the identification of a *MYB* protein involved in the control of vegetative development within subgroup 14 suggests that other members (e.g. *AtMYB36* and *AtMYB87*) might also participate in this process. Much work remains to fully characterize the roles of all *MYB* proteins in regulatory networks. This task is complicated by partially overlapping functional redundancies and difficulties in analyzing the functions of transcription factors produced at very low levels. The use of inducible systems in high throughput expression and interaction studies, combined with bioinformatics and systems analyses in *Arabidopsis*, should provide a robust foundation for predicting the roles of *MYB* proteins in regulatory networks and inferring functions in other plant species.

Acknowledgments

We apologize to our colleagues whose works and original articles could not be cited because of space limitations and the request to cite mainly no more than five-year-old references.

Appendix A. Supplementary data

Supplementary material associated with this article can be found at [doi:10.1016/j.tplants.2010.06.005](https://doi.org/10.1016/j.tplants.2010.06.005)

References

- Paz-Ares, J. *et al.* (1987) The regulatory *c1* locus of *Zea mays* encodes a protein with homology to *myb* proto-oncogene products and with structural similarities to transcriptional activators. *The EMBO Journal* 6, 3553–3558
- Stracke, R. *et al.* (2001) The *R2R3-MYB* gene family in *Arabidopsis thaliana*. *Curr. Opin. Plant Biol.* 4, 447–456
- Du, H. *et al.* (2009) Biochemical and molecular characterization of plant *MYB* transcription factor family. *Biochemistry (Mosc)* 74, 1–11
- Ogata, K. *et al.* (1996) The cavity in the hydrophobic core of Myb DNA-binding domain is reserved for DNA recognition and trans-activation. *Nat. Struct. Biol.* 3, 178–187
- Jia, L. *et al.* (2004) Evolutionary dynamics of the DNA-binding domains in putative *R2R3-MYB* genes identified from rice subspecies *indica* and *japonica* genomes. *Plant Physiol.* 134, 575–585
- Ito, M. (2005) Conservation and diversification of three-repeat Myb transcription factors in plants. *Journal of Plant Research* 118, 61–69
- Haga, N. *et al.* (2007) *R1R2R3-Myb* proteins positively regulate cytokinesis through activation of *KNOLLE* transcription in *Arabidopsis thaliana*. *Development* 134, 1101–1110
- Rosinski, J.A. and Atchley, W.R. (1998) Molecular evolution of the Myb family of transcription factors: evidence for polyphyletic origin. *Journal of Molecular Evolution* 46, 74–83
- Pesch, M. and Hulskamp, M. (2009) One, two, three. models for trichome patterning in *Arabidopsis*? *Curr. Opin. Plant Biol.* 12, 587–592
- Simon, M. *et al.* (2007) Distinct and overlapping roles of single-repeat *MYB* genes in root epidermal patterning. *Dev. Biol.* 311, 566–578
- Dubos, C. *et al.* (2008) *MYBL2* is a new regulator of flavonoid biosynthesis in *Arabidopsis thaliana*. *Plant J* 55, 940–953
- Matsui, K. *et al.* (2008) *AtMYBL2*, a protein with a single *MYB* domain, acts as a negative regulator of anthocyanin biosynthesis in *Arabidopsis*. *Plant J* 55, 954–967
- Lu, S.X. *et al.* (2009) *CIRCADIAN CLOCK ASSOCIATED1* and *LATE ELONGATED HYPOCOTYL* function synergistically in the circadian clock of *Arabidopsis*. *Plant phy.* 150, 834–843
- Hosoda, K. *et al.* (2002) Molecular structure of the *GARP* family of plant Myb-related DNA binding motifs of the *Arabidopsis* response regulators. *Plant Cell* 14, 2015–2029
- Kerstetter, R.A. *et al.* (2001) *KANADI* regulates organ polarity in *Arabidopsis*. *Nature* 411, 706–709
- Waters, M.T. *et al.* (2009) *GLK* transcription factors coordinate expression of the photosynthetic apparatus in *Arabidopsis*. *Plant Cell* 21, 1109–1128
- Rubio, V. *et al.* (2001) A conserved *MYB* transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes & development* 15, 2122–2133
- Jiang, C. *et al.* (2004) Ordered origin of the typical two- and three-repeat *Myb* genes. *Gene* 326, 13–22
- Jiang, C. *et al.* (2004) Identification of conserved gene structures and carboxy-terminal motifs in the *Myb* gene family of *Arabidopsis* and *Oryza sativa* L. ssp. *indica*. *Gen. Bio.* 5, R46
- Wilkins, O. *et al.* (2009) Expansion and diversification of the *Populus* *R2R3-MYB* family of transcription factors. *Plant phy.* 149, 981–993
- Dias, A.P. *et al.* (2003) Recently duplicated maize *R2R3 Myb* genes provide evidence for distinct mechanisms of evolutionary divergence after duplication. *Plant Phy.* 131, 610–620
- Yanhui, C. *et al.* (2006) The *MYB* transcription factor superfamily of *Arabidopsis*: expression analysis and phylogenetic comparison with the rice *MYB* family. *Plant Mol. Bio.* 60, 107–124
- Bedon, F. *et al.* (2007) Conifer *R2R3-MYB* transcription factors: sequence analyses and gene expression in wood-forming tissues of white spruce (*Picea glauca*). *BMC Plant Biology* 7, 17
- Matus, J.T. *et al.* (2008) Analysis of the grape *MYB R2R3* subfamily reveals expanded wine quality-related clades and conserved gene structure organization across *Vitis* and *Arabidopsis* genomes. *BMC Plant Biol.* 8, 83
- Martin, C. and Paz-Ares, J. (1997) *MYB* transcription factors in plants. *Trends Genet.* 13, 67–73
- Stracke, R. *et al.* (2007) Differential regulation of closely related *R2R3-MYB* transcription factors controls flavonol accumulation in different parts of the *Arabidopsis thaliana* seedling. *Plant J* 50, 660–677
- Gonzalez, A. *et al.* (2008) Regulation of the anthocyanin biosynthetic pathway by the *TTG1/bHLH/Myb* transcriptional complex in *Arabidopsis* seedlings. *Plant J* 53, 814–827
- Lepiniec, L. *et al.* (2006) Genetics and biochemistry of seed flavonoids. *Annu Rev. Plant Biol.* 57, 405–430
- Gonzalez, A. *et al.* (2009) *TTG1* complex *MYBs*, *MYB5* and *TT2*, control outer seed coat differentiation. *Dev. Biol.* 325, 412–421
- Jin, H. *et al.* (2000) Transcriptional repression by *AtMYB4* controls production of UV-protecting sunscreens in *Arabidopsis*. *The EMBO Journal* 19, 6150–6161

- 31 Preston, J. *et al.* (2004) AtMYB32 is required for normal pollen development in *Arabidopsis thaliana*. *Plant J.* 40, 979–995
- 32 Zhou, J. *et al.* (2009) MYB58 and MYB63 are transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in *Arabidopsis*. *Plant Cell* 21, 248–266
- 33 Zhong, R. *et al.* (2008) A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in *Arabidopsis*. *Plant Cell* 20, 2763–2782
- 34 Feng, C. *et al.* (2004) *Arabidopsis* MYB68 in development and responses to environmental cues. *Plant Sci.* 167, 1099–1107
- 35 Zhong, R. *et al.* (2007) The MYB46 transcription factor is a direct target of SND1 and regulates secondary wall biosynthesis in *Arabidopsis*. *Plant Cell* 19, 2776–2792
- 36 Yang, C. *et al.* (2007) *Arabidopsis* MYB26/MALE STERILE35 regulates secondary thickening in the endothecium and is essential for anther dehiscence. *Plant Cell* 19, 534–548
- 37 Newman, L.J. *et al.* (2004) Involvement of the R2R3-MYB, AtMYB61, in the ectopic lignification and dark-photomorphogenic components of the det3 mutant phenotype. *Plant J.* 37, 239–250
- 38 Penfield, S. *et al.* (2001) MYB61 is required for mucilage deposition and extrusion in the *Arabidopsis* seed coat. *Plant Cell* 13, 2777–2791
- 39 Liang, Y.-K. *et al.* (2005) AtMYB61, an R2R3-MYB transcription factor controlling stomatal aperture in *Arabidopsis thaliana*. *Current Biology* 15, 1201–1206
- 40 Gigolashvili, T. *et al.* (2008) HAG2/MYB76 and HAG3/MYB29 exert a specific and coordinated control on the regulation of aliphatic glucosinolate biosynthesis in *Arabidopsis thaliana*. *The New Phytologist* 177, 627–642
- 41 Gigolashvili, T. *et al.* (2007) The R2R3-MYB transcription factor HAG1/MYB28 is a regulator of methionine-derived glucosinolate biosynthesis in *Arabidopsis thaliana*. *Plant J* 51, 247–261
- 42 Gigolashvili, T. *et al.* (2007) The transcription factor HIG1/MYB51 regulates indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. *Plant J* 50, 886–901
- 43 Kang, Y.H. *et al.* (2009) The MYB23 gene provides a positive feedback loop for cell fate specification in the *Arabidopsis* root epidermis. *Plant Cell* 21, 1080–1094
- 44 Kirik, V. *et al.* (2005) Functional diversification of MYB23 and GL1 genes in trichome morphogenesis and initiation. *Development* 132, 1477–1485
- 45 Li, S.F. *et al.* (2009) The *Arabidopsis* MYB5 transcription factor regulates mucilage synthesis, seed coat development, and trichome morphogenesis. *Plant Cell* 21, 72–89
- 46 Jakoby, M.J. *et al.* (2008) Transcriptional profiling of mature *Arabidopsis* trichomes reveals that NOECK encodes the MIXTA-like transcriptional regulator MYB106. *Plant Physiol.* 148, 1583–1602
- 47 Baumann, K. *et al.* (2007) Control of cell and petal morphogenesis by R2R3 MYB transcription factors. *Development* 134, 1691–1701
- 48 Zhang, Y. *et al.* (2009) Characterization of *Arabidopsis* MYB transcription factor gene AtMYB17 and its possible regulation by LEAFY and AGL15. *J. Genet. Genomics* 36, 99–107
- 49 Lai, L.B. *et al.* (2005) The *Arabidopsis* R2R3 MYB proteins FOUR LIPS and MYB88 restrict divisions late in the stomatal cell lineage. *Plant Cell* 17, 2754–2767
- 50 Xie, Z. *et al.* (2010) Regulation of stomatal lineage cell proliferation by the *Arabidopsis* MYB four lips via direct targeting of core cell cycle genes. *Plant Cell* (in press)
- 51 Punwani, J.A. *et al.* (2008) The MYB98 subcircuit of the synergid gene regulatory network includes genes directly and indirectly regulated by MYB98. *Plant J* 55, 406–414
- 52 Cheng, H. *et al.* (2009) Gibberellin acts through jasmonate to control the expression of MYB21, MYB24, and MYB57 to promote stamen filament growth in *Arabidopsis*. *PLoS Genet* 5, e1000440
- 53 Mandaokar, A. and Browse, J. (2009) MYB108 acts together with MYB24 to regulate jasmonate-mediated stamen maturation in *Arabidopsis*. *Plant Physiol.* 149, 851–862
- 54 Zhang, Z.B. *et al.* (2007) Transcription factor AtMYB103 is required for anther development by regulating tapetum development, callose dissolution and exine formation in *Arabidopsis*. *Plant J* 52, 528–538
- 55 Brownfield, L. *et al.* (2009) A plant germline-specific integrator of sperm specification and cell cycle progression. *PLoS Genet.* 5, e1000430
- 56 Millar, A.A. and Gubler, F. (2005) The *Arabidopsis* GAMYB-like genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development. *Plant Cell* 17, 705–721
- 57 Muller, D. *et al.* (2006) Blind homologous R2R3 Myb genes control the pattern of lateral meristem initiation in *Arabidopsis*. *Plant Cell* 18, 586–597
- 58 Keller, T. *et al.* (2006) *Arabidopsis* REGULATOR OF AXILLARY MERISTEMS1 controls a leaf axil stem cell niche and modulates vegetative development. *Plant Cell* 18, 598–611
- 59 Lee, D.K. *et al.* (2009) LATERAL ORGAN FUSION1 and LATERAL ORGAN FUSION2 function in lateral organ separation and axillary meristem formation in *Arabidopsis*. *Development* 136, 2423–2432
- 60 Byrne, M.E. *et al.* (2000) Asymmetric leaves1 mediates leaf patterning and stem cell function in *Arabidopsis*. *Nature* 408, 967–971
- 61 Hong, S.H. *et al.* (2008) CRY1 inhibits COP1-mediated degradation of BIT1, a MYB transcription factor, to activate blue light-dependent gene expression in *Arabidopsis*. *Plant J* 55, 361–371
- 62 Yang, S.W. *et al.* (2009) FAR-RED ELONGATED HYPOCOTYL1 and PHY1-LIKE associate with the *Arabidopsis* transcription factors LAF1 and HFR1 to transmit phytochrome A signals for inhibition of hypocotyl elongation. *Plant Cell* 21, 1341–1359
- 63 Wang, X. *et al.* (2008) Overexpression of PGA37/MYB118 and MYB115 promotes vegetative-to-embryonic transition in *Arabidopsis*. *Cell Res.* 19, 224–235
- 64 Mu, R.-L. *et al.* (2009) An R2R3-type transcription factor gene AtMYB59 regulates root growth and cell cycle progression in *Arabidopsis*. *Cell Res.* 19, 1291–1304
- 65 Shin, R. *et al.* (2007) The *Arabidopsis* transcription factor MYB77 modulates auxin signal transduction. *Plant Cell* 19, 2440–2453
- 66 Li, L. *et al.* (2009) *Arabidopsis* MYB30 is a direct target of BES1 and cooperates with BES1 to regulate brassinosteroid-induced gene expression. *Plant J* 58, 275–286
- 67 Raffaele, S. *et al.* (2008) A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in *Arabidopsis*. *Plant Cell* 20, 752–767
- 68 Cominelli, E. *et al.* (2005) A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. *Curr. Biol.* 15, 1196–1200
- 69 Seo, P.J. *et al.* (2009) The MYB96 transcription factor mediates abscisic acid signaling during drought stress response in *Arabidopsis*. *Plant Physiol.* 151, 275–289
- 70 Seo, P.J. and Park, C.M. (2010) MYB96-mediated abscisic acid signals induce pathogen resistance response by promoting salicylic acid biosynthesis in *Arabidopsis*. *The New phy.* 186, 471–483
- 71 Jung, C. *et al.* (2008) Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic *Arabidopsis*. *Plant Physiol.* 146, 623–635
- 72 Reyes, J.L. and Chua, N.H. (2007) ABA induction of miR159 controls transcript levels of two MYB factors during *Arabidopsis* seed germination. *Plant J* 49, 592–606
- 73 Agarwal, M. *et al.* (2006) A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. *J. Biol. Chem.* 281, 37636–37645
- 74 Abe, H. *et al.* (2003) *Arabidopsis* AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell* 15, 63–78
- 75 Devaiah, B.N. *et al.* (2009) Phosphate starvation responses and gibberellic acid biosynthesis are regulated by the MYB62 transcription factor in *Arabidopsis*. *Mol Plant* 2, 43–58
- 76 Mengiste, T. *et al.* (2003) The BOTRYTIS SUSCEPTIBLE1 gene encodes an R2R3MYB transcription factor protein that is required for biotic and abiotic stress responses in *Arabidopsis*. *Plant Cell* 15, 2551–2565
- 77 De Vos, M. *et al.* (2006) The *Arabidopsis thaliana* transcription factor AtMYB102 functions in defense against the insect herbivore *Pieris rapae*. *Plant Signal Behav.* 1, 305–311
- 78 Cominelli, E. *et al.* (2008) Over-expression of the *Arabidopsis* AtMYB41 gene alters cell expansion and leaf surface permeability. *Plant J* 53, 53–64
- 79 Lippold, F. *et al.* (2009) AtMyb41 regulates transcriptional and metabolic responses to osmotic stress in *Arabidopsis*. *Plant Physiol.* 149, 1761–1772

- 80 Van der Ent, S. *et al.* (2008) MYB72 is required in early signaling steps of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Plant Physiol.* 146, 1293–1304
- 81 Segarra, G. *et al.* (2009) MYB72, a node of convergence in induced systemic resistance triggered by a fungal and a bacterial beneficial microbe. *Plant Biol. (Stuttg)* 11, 90–96
- 82 Allen, R.S. *et al.* (2007) Genetic analysis reveals functional redundancy and the major target genes of the *Arabidopsis* miR159 family. *Proc. Natl. Acad. Sci. U. S. A.* 104, 16371–16376
- 83 Addo-Quaye, C. *et al.* (2008) Endogenous siRNA and miRNA targets identified by sequencing of the *Arabidopsis* degradome. *Current Biology* 18, 758–762
- 84 Rajagopalan, R. *et al.* (2006) A diverse and evolutionarily fluid set of microRNAs in *Arabidopsis thaliana*. *Genes & Development* 20, 3407–3425
- 85 Lobbes, D. *et al.* (2006) SERRATE: a new player on the plant microRNA scene. *EMBO Rep.* 7, 1052–1058
- 86 Ogata, K. *et al.* (1995) Comparison of the free and DNA-complexed forms of the DNA-binding domain from c-Myb. *Nat. Struct. Biol.* 2, 309–319
- 87 Heim, M.A. *et al.* (2003) The basic helix-loop-helix transcription factor family in plants: a genome-wide study of protein structure and functional diversity. *Mol. Biol. Evol.* 20, 735–747
- 88 Zimmermann, I.M. *et al.* (2004) Comprehensive identification of *Arabidopsis thaliana* MYB transcription factors interacting with R/B-like BHLH proteins. *The Plant Journal* 40, 22–34
- 89 Grotewold, E. *et al.* (2000) Identification of the residues in the Myb domain of maize C1 that specify the interaction with the bHLH cofactor R. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13579–13584
- 90 Wester, K. *et al.* (2009) Functional diversity of R3 single-repeat genes in trichome development. *Development* 136, 1487–1496
- 91 Baudry, A. *et al.* (2004) TT2, TT8 and TTG1 synergistically specify the expression of BANYULS and proanthocyanidin biosynthesis in *Arabidopsis thaliana*. *Plant J.* 39, 366–380
- 92 Araki, S. *et al.* (2004) Mitotic cyclins stimulate the activity of c-Myb-like factors for transactivation of G2/M phase-specific genes in tobacco. *J. Biol. Chem.* 279, 32979–32988
- 93 Morse, A.M. *et al.* (2009) Post-translational modification of an R2R3-MYB transcription factor by a MAP Kinase during xylem development. *The New phytologist* 183, 1001–1013
- 94 Daniel, X. *et al.* (2004) CK2 phosphorylation of CCA1 is necessary for its circadian oscillator function in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 101, 3292–3297
- 95 Myrset, A.H. *et al.* (1993) DNA and redox state induced conformational changes in the DNA-binding domain of the Myb oncoprotein. *EMBO J* 12, 4625–4633
- 96 Heine, G.F. *et al.* (2004) Two cysteines in plant R2R3 MYB domains participate in REDOX-dependent DNA binding. *J. Biol. Chem.* 279, 37878–37885
- 97 Falcone Ferreyra, M. *et al.* (2010) Cloning and characterization of a UV-B inducible maize flavonol synthase. *The Plant Journal* 62, 77–91
- 98 Serpa, V. *et al.* (2007) Inhibition of AtMYB2 DNA-binding by nitric oxide involves cysteine S-nitrosylation. *Biochemical and biophysical research communications* 361, 1048–1053
- 99 Liu, B. and Shuai, K. (2008) Regulation of the sumoylation system in gene expression. *Curr. Opin. Cell Biol.* 20, 288–293
- 100 Kodadek, T. *et al.* (2006) Keeping transcriptional activators under control. *Cell* 127, 261–264
- 101 Salghetti, S.E. *et al.* (2001) Regulation of transcriptional activation domain function by ubiquitin. *Science* 293, 1651–1653
- 102 Salghetti, S.E. *et al.* (2000) Functional overlap of sequences that activate transcription and signal ubiquitin-mediated proteolysis. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3118–3123
- 103 Seo, H.S. *et al.* (2003) LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. *Nature* 423, 995–999
- 104 Jang, I.C. *et al.* (2007) Independent and interdependent functions of LAF1 and HFR1 in phytochrome A signaling. *Genes & Dev.* 21, 2011–2100
- 105 Molvaersmyr, A.K. *et al.* A SUMO-regulated activation function controls synergy of c-Myb through a repressor-activator switch leading to differential p300 recruitment. *Nucleic Acids Res* in press
- 106 Miura, K. *et al.* (2005) The *Arabidopsis* SUMO E3 ligase SIZ1 controls phosphate deficiency responses. *Proc. Natl. Acad. Sci. U. S. A.* 102, 7760–7765
- 107 Verger, A. *et al.* (2003) Modification with SUMO. A role in transcriptional regulation. *EMBO Rep.* 4, 137–142
- 108 Zheng, Y. *et al.* (2009) Global identification of targets of the *Arabidopsis* MADS domain protein AGAMOUS-Like15. *Plant Cell* 21, 2563–2577
- 109 Palaniswamy, K. *et al.* (2006) AGRIS and AtRegNet: a platform to link cis-regulatory elements and transcription factors into regulatory networks. *Plant Physiol.* 140, 818–829
- 110 Morohashi, K. and Grotewold, E. (2009) A systems approach reveals regulatory circuitry for *Arabidopsis* trichome initiation by the GL3 and GL1 selectors. *PLoS Genet* 5, e1000396
- 111 Koshino-Kimura, Y. *et al.* (2005) Regulation of CAPRICE transcription by MYB proteins for root epidermis differentiation in *Arabidopsis*. *Plant Cell Physiol.* 46, 817–826
- 112 Bailey, P.C. *et al.* (2008) IT3F: a web-based tool for functional analysis of transcription factors in plants. *Phytochemistry* 69, 2417–2425