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Review

Transcriptional regulation of secondary metabolite biosynthesis in plants

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ABSTRACT

Plants produce thousands of secondary metabolites (a.k.a. specialized metabolites) of diverse chemical nature. These compounds play important roles in protecting plants under adverse conditions. Many secondary metabolites are valued for their pharmaceutical properties. Because of their beneficial effects to health, biosynthesis of secondary metabolites has been a prime focus of research. Many transcription factors have been characterized for their roles in regulating biosynthetic pathways at the transcriptional level. The emerging picture of transcriptional regulation of secondary metabolite biosynthesis suggests that the expression of activators and repressors, in response to phytohormones and different environmental signals, forms a dynamic regulatory network that fine-tune the timing, amplitude and tissue specific expression of pathway genes and the subsequent accumulation of these compounds. Recent research has revealed that some metabolic pathways are also controlled by posttranscriptional and posttranslational mechanisms. This review will use recent developments in the biosynthesis of flavonoids, alkaloids and terpenoids to highlight the complexity of transcriptional regulation of secondary metabolite biosynthesis.

1. Introduction

Plants produce thousands of organic compounds that are traditionally divided into two large classes—primary and secondary metabolites. Primary metabolites are essential for plant growth and development and the majority appears to be common in all plants. Secondary metabolites often act as defense molecules and protect plants in various adverse conditions and were once thought to be non-essential for plant growth and development. Our current knowledge has blurred the distinction between these two classes of metabolites, and many researchers now prefer the term "specialized metabolites" to describe secondary metabolites. Plant secondary metabolites are diverse in chemical nature. Biosynthesis of secondary metabolites starts from basic pathways, such as the glycolysis or shikimic acid pathways, and subsequently diversifies, largely depending on cell type, developmental stage and environmental cues. Based on chemical composition, secondary metabolites are broadly divided into two groups: nitrogen-containing molecules (alkaloids) and nitrogen-deficient molecules (terpenoids and phenolics).

Alkaloids are nitrogen-containing molecules mostly derived from amino acids such as tryptophan, tyrosine, phenylalanine and lysine, as well as ornithine [1]. Alkaloids, including terpenoid indole alkaloids (e.g. vinblastine, vincristine), tropane alkaloids (cocaine, scopolamine), and purine alkaloids (caffeine), are known to protect plants from microbial or herbivore attack and from UV-radiation. Many alkaloids produced by plants are of high pharmaceutical value and have been used for the treatment of terminal diseases.

Terpenoids are derived from the universal five-carbon precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Terpenoids play diverse biological functions and are synthesized either in the cytosol, through the mevalonate pathway, or in chloroplasts, through the methylerythritol phosphate (MEP) pathway [2]. Some terpenes act as hormones (e.g. gibberellins—diterpene, brassinosteroids—triterpenes) and have roles in growth and development. Terpenes such as limonene and menthol, act as defensive agents against herbivores.

Phenolics typically have an aromatic ring with a hydroxyl group attached to it. Biosynthesis of phenolics relies upon two pathways, the shikimic acid pathway and the malonic acid pathway. Flavonoids and lignins are important members of this group. In addition to providing beautiful pigmentation in flowers, flavonoids are important in UV-protection, attracting pollinators and seed dispersal. Moreover, these compounds have significant beneficial effects on human health [3,4].

The synthesis and proper accumulation of secondary metabolites are strictly controlled in a spatial and temporal manner and influenced by a number of biotic and abiotic factors. The spatio-temporal transcriptional regulation of metabolic pathways is controlled by a complex network involving many regulatory proteins known as transcription factors (TFs). TFs are sequence specific DNA binding proteins that recognize specific *cis*-regulatory sequences in the promoters of target genes and activate or repress their expression in response to developmental and/or other environmental cues. Some TFs do not bind DNA but interact with other co-factors to form complexes that regulate the expression of the target genes [5]. Recent research has revealed that posttranscriptional

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and posttranslational mechanisms also play significant roles in the regulation of metabolic pathways [6–10]. This review discusses some recent developments in the regulation of flavonoid, alkaloid and terpenoid biosynthesis in plants.

2. Flavonoids (anthocyanins)

Flavonoid biosynthesis starts with the amino acid phenylalanine and the end products include anthocyanins, flavones/isoflavones and condensed tannins (proanthocyanidins, PAs). The majority of flavonoids are conserved among plant species. In the initial steps of the flavonoid pathway, phenylalanine is metabolized to yield coumaroyl-CoA by a series of enzymatic reactions. CHALCONE SYNTHASE (CHS) catalyzes the production of naringenin chalcone by combining one coumaroyl CoA molecule with three malonyl CoA molecules. Chalcone is then isomerized to flavanone by CHALCONE ISOMERASE (CHI) and from this step onward, the pathway diverges to form different classes of flavonoids. In the next step, flavanones are converted to dihydroflavonols by FLAVANONE 3-HYDROXYLASE (F3H). DIHYDROFLAVONOL REDUCTASE (DFR) catalyzes the reduction of dihydroflavonols to flavan-3,4-diols (leucoanthocyanins), which are then converted to anthocyanins through a series of enzymatic steps (Fig. 1a). Most of the structural genes in the anthocyanin biosynthesis pathway are coordinately regulated by a ternary complex comprising of three groups of transcription factors (TFs), namely R2R3 MYB, basic helix–loop–helix (bHLH) and WD-repeat (WDR) proteins [11–14] (Fig. 1a). Most of these regulatory

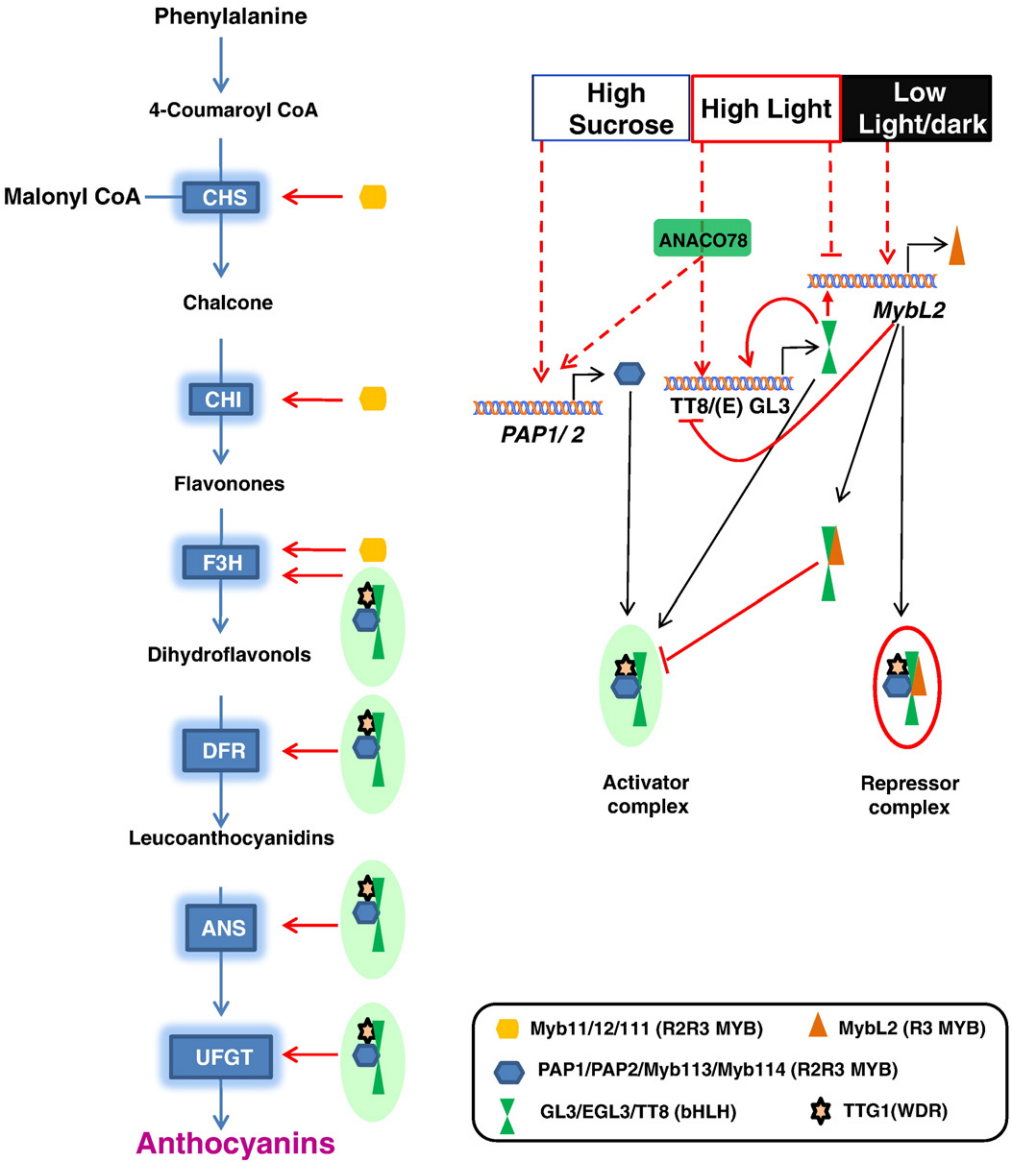

Fig. 1. a. Schematic presentation of gene regulation of anthocyanin biosynthesis in response to environmental and developmental signals in *Arabidopsis*. Structural genes encoding the pathway enzymes are shown in blue boxes. Solid red arrows and T-bars represent the direct activation and repression, respectively. Dotted red arrows and T-bars indicate indirect activation and repression, respectively. Half-circle arrow indicates auto-activation. The activation of pathway genes by the Myb–bHLH–WDR complex (green elliptical circle) and by Myb11/12/111 (solid yellow rectangle) is shown. The red elliptical circle represents the Myb–bHLH–WDR repressor complex. b. Schematic presentation of posttranscriptional and posttranslational regulation of anthocyanin biosynthesis in *Arabidopsis*. Structural genes encoding the pathway enzymes are shown in blue boxes. Solid red arrows and T-bars represent direct activation and repression, respectively. Dotted red arrows and T-bars indicate indirect activation and repression, respectively. The blue shaded area shows the small RNA-mediated regulation of anthocyanin biosynthesis in response to the environmental and developmental signals. The gray shaded area represents posttranslational degradation of components of the Myb–bHLH–WDR complex and Jasmonate ZIM domain (JAZ) proteins by the 26S ubiquitin proteasome system. COP1, CONSTITUTIVE PHOTOMORPHOGENESIS PROTEIN 1; EGL3, ENHANCER OF GLABROUS 3; GL3, GLABROUS 3; TT8, TRANSPARENT TESTA8; TTG1, TRANSPARENT TESTA GLABRA 1; JAZ, JASMONATE ZIM-DOMAIN PROTEIN; PAP1/PAP2, PRODUCTION OF ANTHOCYANIN PIGMENT 1/2; SCF^{COI} complex, SKP-like-CULLIN-F-Box Protein/CORONATINE INSENTITIVE; SPL9, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9; UPL3, UBIQUITIN PROTEIN LIGASE 3.

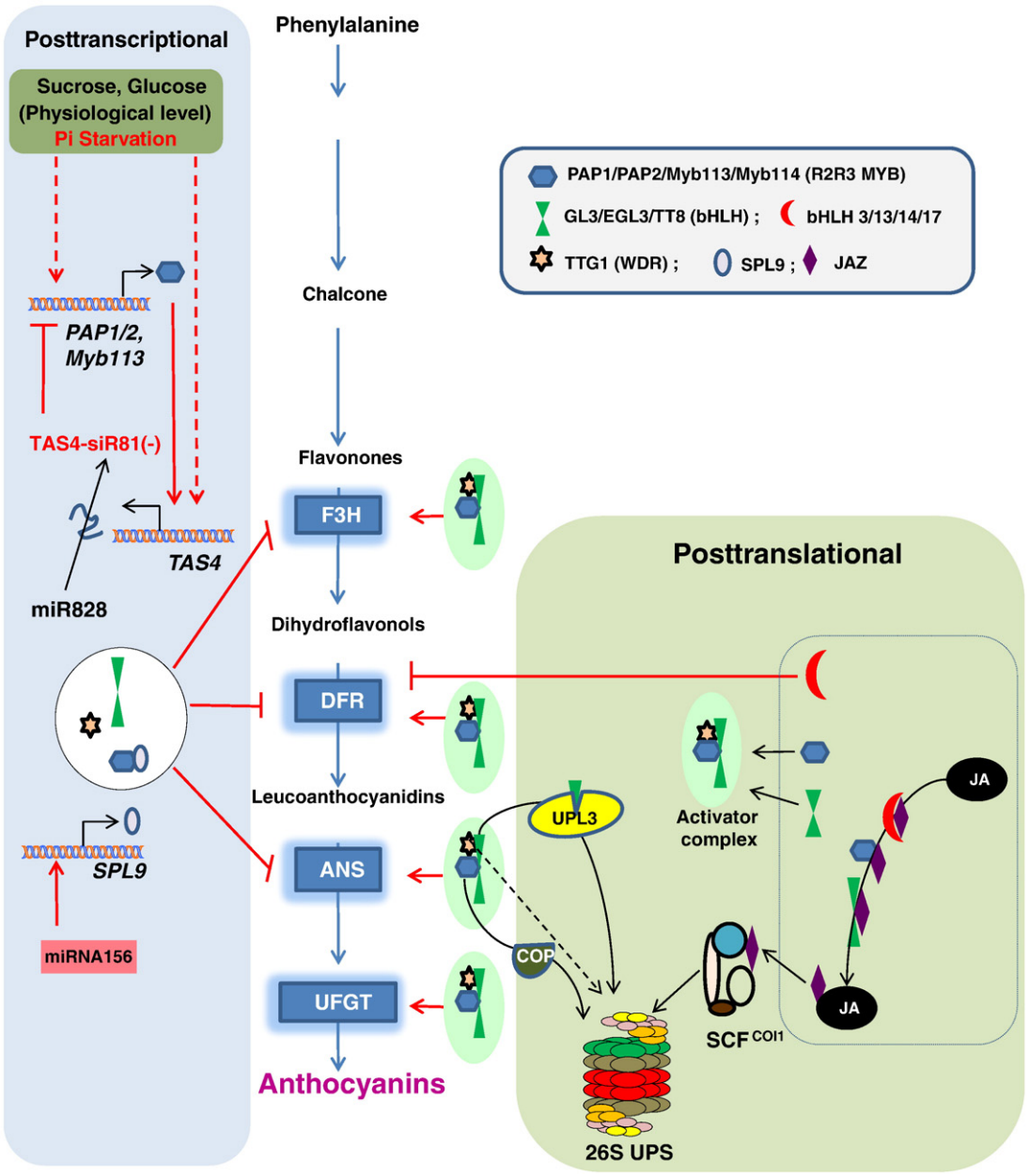

Fig. 1 (*continued*).

proteins have been demonstrated to be functionally conserved among plant species. The coordinated expression of anthocyanin pathway genes by MYB–bHLH–WDR (MBW) complexes exemplifies combinatorial gene regulation in plants. TFs belonging to these three groups have been cloned and characterized from a wide range of plant species (Supplemental Table 1). The current review focuses on a few model and non-model plant species in which the anthocyanin pathway has been well studied, and will highlight recent developments related to pathway regulation.

2.1. Arabidopsis

In *Arabidopsis*, the early biosynthetic pathway genes, such as *CHS*, *CHI*, and *F3H*, are positively regulated by three functionally redundant R2R3 MYB TFs, MYB11, MYB12 and MYB111. Most of the late pathway genes (*DFR*, *ANS*, *UFGT*), however, are regulated by a MBW complex comprised of R2R3 MYBs, MYB75 (PRODUCTION OF ANTHOCYANIN PIGMENTS1; PAP1)/MYB90(PAP2)/MYB113/114, the bHLH factors, GLABROUS3(GL3)/ENHANCER OF GLABROUS3 (EGL3)/TRANSPARENT TESTA8 (TT8), and the WDR protein, TRANSPARENT TESTA GLABRA1 (TTG1) (Fig. 1a) [15,16]. The bHLH TFs, GL3, EGL3 and TT8 play partially redundant roles in control of the anthocyanin pathway. EGL3 plays a major role in activation of late anthocyanin biosynthesis genes [16].

TFs, apart from R2R3 MYBs, bHLH and WDR proteins, have also been shown to regulate anthocyanin biosynthesis in *Arabidopsis*. The single repeat MYBs (R3-MYBs), CAPRICE (CPC) and MYBL2, are reported as negative regulators of the anthocyanin biosynthesis pathway in *Arabidopsis* [17–19]. Both CPC, which lack the R2 domain, and MYBL2, which has a truncated R2 domain, have the R3 domain that contains a protein–binding interface. They compete with the R2R3 MYBs (PAP1/PAP2) for binding to the bHLH regulators (GL3/EGL3/TT8) and inhibit the formation of an active MBW complex. A six amino acid motif (TLLLFR) present in the C-terminal domain of MYBL2 has been identified as a novel repressor motif that is different from the ERF-associated amphiphilic repression (EAR) motif found in PhMYB27 or FaMYB1, isolated from petunia and strawberry, respectively [20].

Anthocyanin biosynthesis is regulated by multiple factors including light, sugar and phytohormones [21,22]. In *Arabidopsis*, MYBL2 has been proposed to play a key regulatory role in the induction of anthocyanin accumulation by high light and sucrose conditions. Expression of MYBL2 is induced under low light or stress-free conditions and the accumulated protein interacts with bHLH factors (GL3/EGL3/TT8) to form an inactive complex that suppresses pathway gene expression and subsequent anthocyanin accumulation. Conversely, high light or stress conditions suppress expression of MYBL2 and induce expression of PAP1 and TT8, resulting in the formation of an active MBW complex, and the ensuing up-regulation of pathway genes induces anthocyanin biosynthesis [17,18]. It has also been shown that TT8 and MYBL2 are in a transcriptional regulatory loop in which TT8 is an activator of MYBL2 that negatively regulates *TT8* expression [18]. These observations highlight the complexity of the regulatory network that controls and fine-tunes the accumulation of these compounds.

Recently, the NAC domain TF, ANAC078, has also been shown to be involved in transcriptional activation of anthocyanin pathway genes in high light conditions. Upon perception of high light signals, ANAC078 rapidly accumulates in cells and up-regulates more than 165 genes, including *PAP1*, which triggers the anthocyanin biosynthesis pathway. These phenomena were further confirmed by analyzing overexpression and knockout *ANAC078* plants that were affected in anthocyanin accumulation after being exposed to high light stress [23]. Some of the regulators in the *Arabidopsis* flavonoid pathway are also involved in trichome development. The first evidence of this relationship comes from analysis of the *ttg1* mutant that is glabrous and defective in pigment accumulation. Other factors involved in both process include the bHLH factors, GL3/EGL3, and the R3 MYB, CPC. The *gl3egl3* double mutant is also defective in both processes. Moreover, both flavonoid synthesis and trichome development in *Arabidopsis* are regulated by the same MBW complex. These findings underscore the possible co-evolution of the metabolic and developmental pathways in *Arabidopsis* [11].

2.2. Petunia

In petunia, the MBW complex comprised of ANTHOCYANIN2 (AN2)/AN4 (R2R3 MYB), AN1 (bHLH) and AN11 (WDR), regulates the late anthocyanin biosynthesis genes [24–27]. AN2 is involved in color development in petal limb, whereas AN4 controls color in the petal tube and anthers [24]. JAF13, another bHLH TF, is also involved in flower color development in petunia, however, it is not functionally redundant with AN1 [28]. In addition to AN2 and AN4, several other MYB TFs have been recently shown to be involved in petunia anthocyanin biosynthesis [29]. The R2R3 MYB factors, DEEP PURPLE (DPL) and PURPLE HAZE (PHZ), isolated from leaf tissues, regulate anthocyanin biosynthesis in vegetative and floral tissues, and work coordinately with AN1 and AN11 [29]. PHZ is significantly induced under high light conditions and is the predominant regulator of vegetative tissue pigmentation in petunia. DPL, on the other hand, is moderately induced by light and is thought to make a smaller contribution to pigmentation in vegetative tissues. In flowers, DPL regulates the vein-associated pigmentation of the flower tube, whereas PHZ contributes to light-induced anthocyanin accumulation on the exposed petal surface [29]. In petunia, the R2R3-MYB, PhMYB27, acts as a negative regulator of anthocyanin biosynthesis. It has an EAR-type repression motif at the carboxy terminus, similar to FaMYB1 of strawberry [20,29]. PhMYB27 can bind the bHLH factor AN1, and repress anthocyanin synthesis. *PhMYB27* is highly expressed in shade-grown leaves and is repressed by high light, whereas expression of *PHZ*, *DPL* and *AN1* are up-regulated under high light resulting in higher anthocyanin accumulation in vegetative tissues [29]. The similarities between light-induced expression patterns of activators and repressors in petunia and *Arabidopsis*, suggest that the mechanism controlling anthocyanin synthesis in vegetative tissues is conserved among these plants. The R3-MYB, PhMYBx, a petunia homolog of CPC in *Arabidopsis*, also acts as a negative regulator of anthocyanin accumulation in petunia [29].

2.3. Tobacco

In tobacco, anthocyanin accumulation in flowers is regulated by the bHLH and R2R3 MYB TFs, NtAN1 and NtAN2, respectively. The NtAN1–NtAN2 complex strongly activates the late pathway genes, and moderately activates early pathway genes. Overexpression of *NtAN2* in tobacco results in increased pigment accumulation in floral and vegetative tissues, whereas pigmentation in *NtAN1*-overexpression lines is restricted only to flowers [30,31].

2.4. Snapdragon

In Snapdragon, the R2R3 MYBs, ROSEA1, ROSEA2 and VENOSA control floral anthocyanin accumulation through interaction with bHLH factors, DELILA or MUTABILIS [32]. The intensity and pattern of anthocyanin pigmentation in flowers are controlled by the MYB factors.

2.5. Maize

In maize, the anthocyanin pathway is regulated through the cooperation of the R2R3 MYB factors, C1/PL, with R/B (bHLH) and PAC1 (WD40) [33–37]. In addition to an N-terminal MYB-interaction domain and a bHLH domain, the bHLH TF R, contains a C-terminal protein–protein interaction domain that shares structural similarity to the ACT domain previously found in some metabolic enzymes. Dimerization of the ACT domain has been shown to be important for anthocyanin biosynthesis in maize [38]. Structural homology modeling has identified other bHLH TFs with ACT-like domains; however, the significance of this domain in the regulatory activities of these factors remains to be determined. Recently, an AGENET domain-containing EMSY-like nuclear factor, known as R-Interacting Factor 1 (RIF-1), has been added to the list of anthocyanin regulators in maize. EMSY-like factors are known to be involved in chromatin remodeling. RIF-1 was isolated using the C-terminal region of R in a yeast two hybrid screen and shown to specifically interact with the bHLH domain of R [39]. The function of the bHLH domain in R/B is unknown so far. Previous studies have shown that this domain is dispensable for activation of anthocyanin pathway promoters in transient assays and no proteins that interact with this domain have been reported [34]. Identification of RIF-1 as a bHLH domain interacting partner of R suggests a unique role for this conserved domain. Chromatin immunoprecipitation (ChIP) and transient expression assays in maize cells further demonstrated that RIF-1 is preferentially associated with C1-R mediated regulation of the *A1* (*DFR*) promoter. These findings linking transcriptional activation with chromatin remodeling, establish a novel function for the bHLH domain of R [39]. Although AGENET domain-containing EMSY-like factors are present in other plants, including *Arabidopsis*, their interaction with bHLH regulators of MBW complexes and role in anthocyanin biosynthesis remains to be elucidated.

2.6. Fruits (apple, grape and strawberry)

The color in fruit skin and pulp are a rich source of anthocyanin and anthocyanidin glycosides that have significant beneficial effects on human health. TFs orthologous to the MBW complex of *Arabidopsis* have been isolated from a number of fruits including apple, pear, peach, plum, strawberry and grapes, and their roles in fruit skin and flesh color were investigated [40–42]. In apple, *MdMYB1*, *MdMYB10* and *MdMYBA*, which are allelic to each other, act as activators of anthocyanin synthesis in skin and flesh when co-expressed with bHLH factors, MdbHLH3 and MdbHLH33 [40,41,43]. A set of R2R3 MYBs (VvMYBA1/2/5a/5b), bHLH (VvMYC1, VvMYCA1), and WD40 (VvWDR1 and VvWDR2) proteins, isolated from grape, are implicated in

anthocyanin biosynthesis [44–46]. In strawberry, expression of two R2R3 MYBs, *FaMYB10* and *FaMYB1*, has been shown to be associated with fruit development. Expression of both of these *MYBs* increases significantly upon ripening and color change, suggesting their involvement in anthocyanin accumulation in fruit [42]. FaMYB10 is an activator of anthocyanin biosynthesis, whereas FaMYB1 acts as a repressor. The C-terminus of FaMYB1 contains a repressor domain similar to PhMYB27 of petunia, and overexpression of FaMYB1 in tobacco severely affects floral accumulation of anthocyanin [20]. High-level expression of both an activator and a repressor at the fruit ripening stage indicates that they may serve to fine-tune the expression of pathway genes thereby balancing the accumulation of anthocyanin pigments in fruits.

2.7. Regulation of the regulators of flavonoid biosynthesis

Regulation of structural genes in flavonoid biosynthesis pathway is well documented in a wide range of plants. However, information pertaining to what regulates their transcriptional regulators is limited. Recent studies on a few model plant species provided useful information about the regulatory network. The R2R3 MYB TF, AN2, and bHLH factor, AN1, are positive regulators of the anthocyanin pathway in petunia and tobacco. Overexpression of *AN2* results in a significant accumulation of *AN1* transcripts in leaves, a tissue that does not normally express *AN1*, suggesting that *AN1* is regulated by AN2 [24,30]. Additionally, in petunia, *AN1* expression in anthers is controlled by another MYB factor, AN4 [24]. The expression of *VvMYC1*, which encodes a bHLH factor involved in anthocyanin and PA accumulation in grapevine, is regulated by a complex of VvMYC1 and VvMYBPA1 (a PA-specific R2R3 MYB) [46]. In *Arabidopsis*, the regulation of the bHLH factor TT8, that regulates both anthocyanin and PA accumulation, has been studied in detail [47]. Functional analysis of the *TT8* promoter has revealed two important modules which are sufficient for driving its expression in anthocyanin and PA accumulating cells, and a third module is responsible for the strength of the promoter. Functional assays of these two modules in different regulatory mutants have shown that *TT8* promoter expression is controlled by at least six different MBW complexes, three of which (PAP1-TT8-TTG1, PAP1-EGL3-TTG1 and PAP1-GL3-TTG1) are responsible for anthocyanin accumulation in the cotyledons' margin whereas the other three (TT2-TT8-TTG1, TT2-EGL3-TTG1 and MYB5-TT8-TTG1) are dedicated to PA-accumulating cells in seeds. Collectively, it appears that in dicots, the TFs in the MBW complex are in a regulatory loop that controls expression of one another in a spatial and temporal manner. However, mutant analysis of anthocyanin regulatory loci in the monocot, maize shows that the TFs in the MBW complex are independently regulated. These observations suggest that, although the function of the TFs in the MBW complex, as regulators of anthocyanin, is conserved in monocots and dicots, the mechanism that regulates these TFs has been reorganized [33].

3. Alkaloids

While transcriptional regulation of flavonoids has been extensively studied, the regulation of alkaloids remains less well characterized. In the following section we discuss the regulation of terpenoid indole alkaloids (TIAs), nicotine, benzylisoquinolines and camalexin.

3.1. Terpenoid indole alkaloids

TIAs are found in a limited number of plant species belonging to the families Apocynaceae, Loganiaceae, Nyssaceae and Rubiaceae. *Catharanthus roseus*, a member of the Apocynaceae family, also commonly known as Madagascar periwinkle, has become a model plant for understanding TIA biosynthesis and regulation [48,49]. TIAs can be characterized into two principle groups: monoterpene indole alkaloids (MIAs), and the bisindole alkaloids, which are composed of two joined MIAs. The primary TIAs of pharmaceutical interest in *Catharanthus* include ajmalicine, serpentine, vinblastine, and vincristine. The MIAs, ajmalicine and serpentine, also present in Indian Serpentwood (*Rauwolfia serpentina*), are utilized pharmaceutically for the treatment of hypertension [50]. The bisindole alkaloids, vinblastine and vincristine, are species specific antineoplastic metabolites which have proven invaluable in the treatment of cancers [51]. The biosynthesis of bisindole alkaloids is notably complex and requires extensive inter- and intracellular transport [52–55]. The biosynthesis of TIAs has been recently reviewed [56–58], and is not covered here.

Several key committed steps in TIA biosynthesis have been the primary targets of studies for transcriptional regulation. As entry points into a pathway, committed steps provide an opportunity for pathway manipulation to increase production of secondary metabolites. In TIA biosynthesis, the committed steps to MIAs include the formation of the tryptamine precursor, formation of the secoiridoid terpene precursor (secologanin), and the coupling of these two precursors into the first TIA, strictosidine. Tryptamine, which contributes the indole moiety of TIAs, is derived from tryptophan by a decarboxylation reaction catalyzed by TRYPTOPHAN DECARBOXYLASE (TDC). Secologanin provides the terpenoid part of TIAs and is formed through a series of enzymatic reactions from geraniol. The first step in secologanin biosynthesis, the conversion of geraniol to 10-hydroxygeraniol, is believed to be the overall rate-limiting step in the TIA biosynthesis. The condensation of secologanin and tryptamine results in the formation of strictosidine, and is catalyzed by the enzyme STRICTOSIDINE SYNTHASE (STR) (Fig. 2) [1].

Studies investigating committed or rate-limiting steps of alkaloid producing species reveal two key families of TFs: the AP2-ERF and WRKY families. AP2/ERF TFs contain a single, 60–70 amino acid, AP2 domain [59,60], and were some of the first TFs identified in the regulation of *Catharanthus* TIAs [61,62] (Supplemental Table 2). WRKY TFs contain a WRKY domain possessing a conserved WRKYGQK motif and a zinc-finger motif [63]. WRKY TFs are known to function in the regulation of plant responses in plant development and, abiotic and biotic responses [64,65], including secondary metabolite production [66–71].

Transcriptional regulation of *TDC* and *STR* has been extensively studied. Expression of *TDC* and *STR* is coordinately induced in response to jasmonic acid (JA) and fungal elicitor treatment. Deletion analysis of *TDC* and *STR* promoters has revealed the presence of several important *cis*-regulatory elements including the jasmonate- and elicitor-responsive elements (JERE). The JERE in the *STR* promoter has been used as bait in yeast one-hybrid assays to isolate two AP2/ERF family members, OCTADECANOID RESPONSIVE CATHARANTHUS AP2-DOMAIN 1 (ORCA1) and ORCA2, from *Catharanthus* [61]. ORCA2 bound the so-called RV region of the *STR* promoter, containing the JERE with a GCC-core, and its expression is rapidly induced by JA and elicitor treatment. ORCA1 also binds to the same region of the *STR* promoter but its expression is not induced by JA treatment. Moreover, transient overexpression of ORCA2 in *Catharanthus* cells, strongly activates the *STR* promoter, whereas ORCA1 has marginal effect on *STR* expression. These findings suggest that ORCA2 plays a crucial role in JA and elicitor responsive expression of *STR*. Another closely related AP2/ERF TF, ORCA3, has been isolated from T-DNA activation tagged cell lines of *Catharanthus* [62]. *ORCA3* expression is rapidly induced by JA, and its overexpression in *Catharanthus* cell lines induced multiple genes in the TIA pathway. Moreover, ORCA3 binds to JA-responsive regions of *STR* and *TDC* promoters, in vitro, and trans-activates their expression in a transient assay, indicating a role as a master regulator of the TIA pathway.

Sequence analysis of several *Catharanthus* TIA pathway promoters, including that of *TDC*, revealed the presence of multiple W-box *cis*-elements, a canonical DNA-binding motif for WRKY TFs. However, until now, the biological significance of those *cis*-elements in TIA pathway promoters was not demonstrated. Recently, a WRKY TF, CrWRKY1, isolated from *Catharanthus* seedlings has been shown to play a crucial role in TIA biosynthesis [71]. *CrWRKY1* is induced by methyl jasmonate

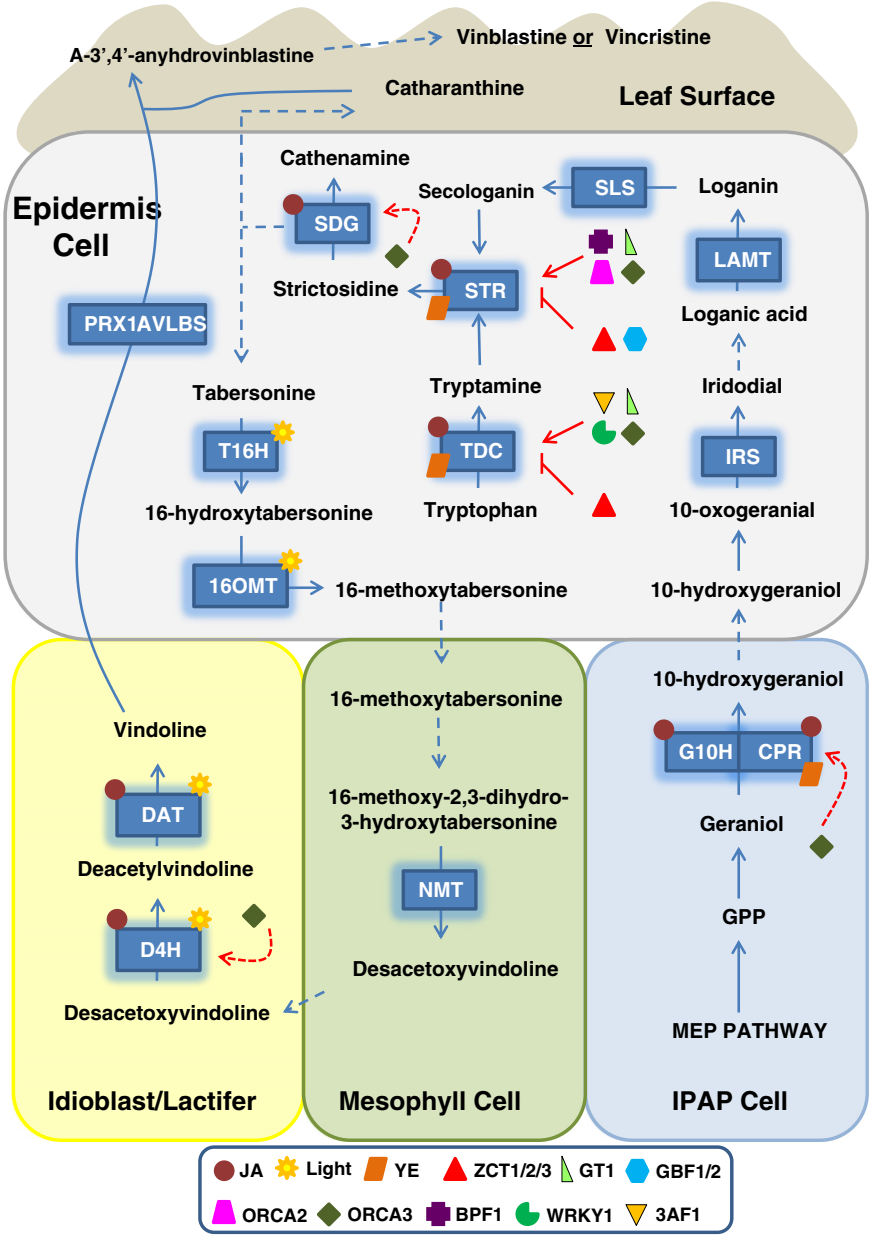

Fig. 2. Schematic presentation of the regulation of Terpenoid Indole Alkaloid (TIA) biosynthesis in *Catharanthus roseus*. Production of TIAs in *C. roseus* is affected by a complex combination of sub-cellular localization, multiple cell types (indicated by different colors), developmental, and spatio-temporal factors. TFs are regulated by abiotic, biotic, or endogenous developmental signals to modulate the production of TIAs. Jasmonate (JA) and yeast extract (YE) elicitor are potent inducers of TIA production by activating multiple TFs. Environmental factors, such as light, have also been demonstrated as important regulators affecting the expression of several genes late in the TIA pathway. Regulatory factors binding to gene promoters function both as activators (ORCA2, ORCA3) and repressors (ZCTs, GBFs) to modulate TIA biosynthetic gene expression. TFs demonstrated to bind to specific TIA biosynthetic gene promoters are depicted. BPF-1, BOX P-BINDING FACTOR 1; CPR, CYTOCHROME P450 REDUCTASE; D4H, DESACETOXYVINDOLINE-4-HYDROXYLASE; DAT, DEACETYLEVINDOLINE 4-O-ACETYLETRANSFERASE; GBF, G-BOX BINDING FACTOR; G10H, GERNIOL 10-HYDROXYLASE; IRS, IRIDOID SYNTHASE; LAMT, LOGANIC ACID METHYLTRANSFERASE; NMT, N-METHYLTRANSFERASE; OMT, *O*-METHYLTRANSFERASE; ORCA, OCTADECANOID RESPONSIVE CATHARANTHUS AP2-DOMAIN; PRX: PEROXIDASE; SLS: SECOLOGANIN SYNTHASE; SGD: STRICTOSIDINE β-D-GLUCOSIDASE; STR, STRICTOSIDINE SYNTHASE; T16H: TABERSONINE 16-HYDROXYLASE; TDC, TRYPTOPHAN DECARBOXYLASE; ZCT, ZINC-FINGER *C. roseus* TRANSCRIPTION FACTOR.

(MeJA), ethylene, and gibberellic acid (GA). Overexpression of *CrWRKY1* in *Catharanthus* hairy roots results in up-regulation of several TIA pathway genes, especially *TDC*, as well as the transcription repressors, *ZCT1* (ZINC-FINGER *C. roseus* TRANSCRIPTION FACTOR 1), *ZCT2* and *ZCT3*. However, the expression of transcriptional activators, *ORCA2*, *ORCA3* and *MYC2*, are reduced. CrWRKY1 binds the W-box motif in the *TDC* promoter and trans-activates the *TDC* promoter in *Catharanthus* cells. Moreover, the transgenic hairy roots accumulate higher amounts of serpentine compared to the control roots. These findings demonstrate the role of WRKY family TFs in the regulation of TIA biosynthesis in *Catharanthus*.

In addition to AP2/ERF and WRKY TFs, the *TDC* and *STR* promoters were also shown to be regulated by several other TFs, including BOX P-BINDING FACTOR 1 (BPF-1), G-BOX BINDING FACTORS (GBF1 and GBF2), and ZCTs [72–74]. BPF-1 is a MYB-like protein that was isolated from *Catharanthus* using a JA and elicitor responsive region, the so-called BA region, of the *STR* promoter in a yeast one-hybrid assay. BPF-1 has high homology to parsley box P-binding factor. *BPF-1* expression is induced by fungal elicitors but remains unchanged in response to JA. These studies indicate that BPF-1 is possibly involved in an elicitor-responsive JA-independent signal transduction pathway in *Catharanthus*.

GBF1 and GBF2 belong to the basic leucine zipper (bZIP) family of TFs, and bind to the G-box (CACGTG) motif in the *STR* promoter. Transient bombardment assays showed that GBF1 and GBF2 act as repressors of the *STR* promoter. ZCT1, ZCT2 and ZCT3 belong to the Cys_2/His_2 (TF IIIA-type) zinc finger protein family, and were isolated by yeast one-hybrid using the elicitor responsive region of the *TDC* promoter. Expression of *ZCT* genes is also induced by yeast extract and MeJA. The ZCT TFs bind to multiple regions of both *TDC* and *STR* promoters. The binding site of ZCTs in the *STR* promoter is distinct but overlaps with binding sites for ORCAs. In a transient assay, ZCT proteins repress the activities of *TDC* and *STR* promoters, suggesting their role as potential transcriptional repressors in the TIA pathway. In addition to JERE, UV-light responsive *cis*-regulatory sequences have been identified in both *TDC* and *STR* promoters. The GT-1 and 3AF1 TFs bind multiple elements in the *TDC* and *STR* promoters to enhance their expression in response to UV-light [75].

Secologanin forms the second moiety necessary for formation of TIAs. The production of secologanin, through the iridoid terpene pathway, starts with the formation of geraniol followed by hydroxylation into 10-hydroxygeraniol, which is catalyzed by the cytochrome P450 enzyme, geraniol 10-hydoxylase (G10H). The cytochrome P450 reductase (CPR) is essential for G10H catalyzed reaction. Increased TIA accumulation by feeding cell cultures with loganin suggests that G10H is a rate limiting step in TIA production [62]. Unlike *TDC* and *STR*, the regulation of other TIA pathway genes is not well studied. Analysis of the *G10H* promoter reveals the presence of several putative DOF, GBF, MYB, and WRKY TF binding sites, indicating that TFs belonging to these families are probably involved in *G10H* regulation [76]. The expression of *CPR* has been shown to be regulated by the AP2/ERF TF, ORCA3 [62].

The formation of vindoline, a precursor to vinblastine and vincristine, requires *DEACETYLEVINDOLINE 4-O ACETYLETRANSFERASE* (*DAT*). DAT transfers an acetyl group onto deacetylvindoline to produce vindoline. JA and light have both been demonstrated as regulators of DAT expression [77,78]. The *DAT* promoter contains *cis*-elements that regulate ABA, auxin, JA, light and defense responses [78,79]. Wang et al. [78] identified three TGACG-motifs and an inverted motif (CGTCA) within the *DAT* promoter that are involved in MeJA-responsive expression.

3.1.1. Regulation of the regulators of TIA biosynthesis

TIA biosynthesis in *Catharanthus* is regulated by TFs belonging to different families. However, the regulation of these TFs has not been well studied. The regulation of *ORCA3*, a critical regulator of TIA biosynthesis, has been a subject for investigation. The *ORCA3* promoter has a 74-bp region containing a bipartite JA responsive element (JRE). The JRE is composed of an A/T-rich quantitative sequence responsible for high-level expression, and a qualitative component with a T/G-box element acting as an on/off switch in response to MeJA. The bipartite JRE of the *ORCA3* promoter has been used in a yeast-one hybrid assay to isolate several AT-hook DNA-binding motif-containing proteins. Two of these AT-hook TFs bind to the A/T-rich quantitative sequence and weakly activates the *ORCA3* promoter, suggesting that they may be important for regulating expression of *ORCA3* in a quantitative manner [80]. A recently characterized bHLH TF, CrMYC2 from *Catharanthus*, is an immediate-early JA-responsive factor that binds to the T/G-box containing the qualitative sequence in the *ORCA3* promoter. CrMYC2 activates the *ORCA3* promoter in a transient assay, and RNAi-mediated suppression of *CrMYC2* significantly affects *ORCA3* and *ORCA2* expression [81]. These findings suggest that MeJA-responsive expression of TIA pathway genes is controlled by a TF cascade, and CrMYC2 acts upstream of the ORCAs. Recently, characterization of the *CrWRKY1* promoter revealed the importance of several new *cis*-elements, including the TGACG motifs, and suggests the involvement of novel TFs in this regulatory network [82].

3.2. Nicotine

Nicotine, the predominant alkaloid of tobacco (*Nicotiana tabacum*), possesses potent insect anti-feedant properties [83]. Upon mechanical or herbivore wounding, nicotine biosynthesis occurs in tobacco roots [84], and is then transported to leaf tissue [85]. Biosynthesis of nicotine is also induced by JA. The NIC2 locus of tobacco contains seven AP2-ERF TFs which, in combination, affect the expression of all nicotine biosynthetic genes [86]. The JA inducible *ORC1*, also known as *ERF221*, and ERF189 *AP2-ERF* TFs are both genes in the NIC2 locus. ORC1 and ERF189 have overlapping but non-redundant roles in regulating nicotine biosynthetic genes [86,87]. ORC1 recognition of the GCC-box of the *PMT* (*PUTRESCINE N-METHYLTRANSFERASE)* promoter was necessary but not sufficient for activation. The bHLH TF, NtMYC2, directly regulates the expression of selected nicotine biosynthetic genes and also regulates nicotine biosynthesis through regulation of the NIC2 AP2-ERF transcription factors [88]. A *N. benthamiana* MYC2 homolog, NbbHLH1, functions, in combination with ORC1, in binding to the G- and GCC-boxes of *PMT1* and *QUINOLINATE PHOSPHORIBOSYLTRANSFERASE (QPRT2)* promoters, respectively; both TFs are necessary for optimal *PMT1* and *QPRT2* activation [87]. Moreover, multiple GCC-boxes are required for the full activation of *QPRT2* by ERF189 [89]. Collectively, these findings indicate the intricate interaction between diverse TFs in the regulation of alkaloids. Future work is needed to determine the roles of the other five NIC2 locus ERFs specifically play in regulating nicotine biosynthesis.

3.3. Benzylisoquinolines

Transcriptional regulation of other classes of alkaloids, such as benzylisoquinolines, synthesized by Opium Poppy (*Papaver somniferum*) and Goldthread (*Coptis japonica*) (Fig. S1), is even more poorly characterized. Kato et al. [68] characterized CjWRKY1, from *C. japonica*, which is involved in regulation of the benzylisoquinoline alkaloid berberine. CjWRKY1 was found to positively regulate the expression of all but the last step of biosynthetic genes leading to berberine formation, but did not impact the expression of genes involved in primary metabolism. CjWRKY1 thus appears to function as a master regulator for berberine biosynthesis. A bHLH family TF from *C. japonica*, CjbHLH1, was also found to regulate all but the last gene involved in berberine biosynthesis [90]. Homologs of CjbHLH1 were found only in species producing isoquinoline alkaloids. Apuya et al. [91] heterologously expressed TFs from *Arabidopsis*, soybean (*Glycine max*), and maize (*Zea mays* ssp. *mays*), to identify regulators of alkaloid biosynthetic genes from Opium Poppy and California Poppy (*Eschscholzia californica*). They identified AtWRKY1 as regulating the expression of multiple alkaloid biosynthetic genes in both Opium and California poppies.

3.4. Camalexin

One of the primary defense metabolites present in *Arabidopsis* is the phytoalexin camalexin. Camalexin is a tryptophan derived sulfur-containing molecule synthesized in response to various pathogen and reactive oxygen species inducing stresses and is the major phytoalexin in *Arabidopsis* [92]. Expression of camalexin biosynthetic genes is governed by bHLH [93], DOF [94], MYB [95], NAC [96] and WRKY [97] TFs. *Pseudomonas syringae* pv. *tomato* and the bacterial elicitor, flagellin, activate a kinase cascade leading to the phosphorylation of MAP KINASE SUBSTRATE 1 (MKS1) which releases WRKY33 from a complex [97,98]. Activated WRKY33 directly binds to the promoter of PHYTOALEXIN DEFICIENT3 (PAD3), CYP71B15, which forms the final step in the synthesis of camalexin [97]. Camalexin production is also regulated by WRKY33 through direct phosphorylation by MPK3 [70]. Interestingly, a MPK3 homolog in *Catharanthus* was recently reported to function in regulation of TIA accumulation [99].

4. Terpenes

Terpenes comprise the largest class of plant secondary metabolites, containing in excess of 50,000 chemicals identified [100], and many biosynthetic enzymes in the pathway have been characterized. Despite the wealth of knowledge regarding the synthesis of terpenes, transcriptional regulation of these compounds is the least well studied, partially due to alternative methods available to increase chemical production [101]. As with alkaloids the AP2-ERF and WRKY transcription factor families are quickly emerging as important regulators of terpene biosynthesis (Supplement Table 3).

Recent advances in Sweet Wormwood (*Artemisia annua*) have uncovered several TFs leading to the biosynthesis of artemisinin, a sequiterpene lactone widely utilized in the treatment of malaria (Fig. S2). Two jasmonate responsive AP2-ERF TFs from *A. annua*, AaERF1 and AaERF2, regulate the transcription of AMORPHA-4,11-DIENE SYNTHASE (ADS) and CYP SEQUITERPENE OXIDASE (CYP71AV1) [102]. Both AaERF1 and AaERF2 belong to the same B3 subfamily of AP2-ERF TFs as *Catharanthus* ORCA2 and ORCA3. ADS and CYP71AV1 are also regulated by the WRKY TF, AaWRKY1. AaWRKY1 further regulates 3-HYDROXY 3-METHYLGLUTARYL-COA REDUCTASE (HMGR) and ARTEMISINIC ALDEHYDE Δ11(13) REDUCTASE (DBR2). Recently, the AP2/ERF TF AaORA1, has been shown to regulate accumulation of artemisinin, and contribute to *A. annua* defense against the necrotrophic plant pathogen, *Botrytis cinerea* [103]. With the exception of HMGR, AaORA1 regulates the same enzymes as AaWRKY1. Collectively, AaERF1, AaERF2, AaORA1 and AaWRKY1 positively regulate all the early steps of artemisinin biosynthesis prior to a branch in the pathway leading to either dihydroartemisinic acid and artemisinin, or artemisinic acid and arteannuin B. AaORA1 and AaWRKY1 also regulate *DBR2*, which catalyzes the conversion of artemisinic aldehyde into dihydroartemisinic acid, directing the pathway towards artemisinin formation. In *Artemisia*, increased production of either artemisinin or artemisinic acid is desirable as a semisynthetic conversion of artemisinic acid to artemisinin is possible [104].

WRKY TFs have also been cloned and characterized from several other terpene producing plants. *Taxus* species provide the valuable anticancer drug paclitaxel. The enzyme 10-DEACETYLBACCATIN III-10 β-O-ACETYL TRANSFERASE (DBAT) is a key rate limiting step in the synthesis of paclitaxel [105]. Overexpression of DBAT was previously shown to increase the accumulation of paclitaxel in cell suspension [106]. Li et al. [67] recently identified a WRKY TF, TaWRKY1, from *Taxus chinensis* cells, as a regulator of DBAT. GaWRKY1, from cotton (*Gossypium arboreum*), regulates the expression of (+)-δ-CADINENE SYNTHASE (CAD1), a branch point in the synthesis of sesquiterpenes leading to gossypol [69]. GaWRKY1 not only regulates CAD1 developmentally in a temporal and spatial manner, but also in response to fungal and jasmonate elicitor treatment in cell suspension cultures [69]. Recently, HbEREBP1 and HbWRKY1, from rubber tree (*Hevea brasiliensis*), have been implicated in regulation of latex production [107,108]. HbWRKY1 was found to be strongly induced by abscisic acid, ethylene, jasmonate, osmotic stress, *Oidium heveae* infection, and wounding [108]. HbEREBP1 may be a negative regulator of early jasmonate and wounding induction of latex biosynthesis [107]. Together, these examples demonstrate that the WRKY transcription factor family clearly plays a prominent role in the regulation of terpenoid compounds.

5. Recent developments in pathway regulation

Studies related to the regulation of secondary metabolite pathways have been focused mainly on characterization of TFs regulating the structural genes. However, only a few have provided mechanistic insight about regulation. Moreover, recent studies indicate that besides transcriptional control, these pathways are also regulated by posttranscriptional and posttranslational control mechanisms.

Coordinated regulation of structural genes by the MBW complex is a hallmark of the anthocyanin pathway. Maize has long been used as a model to study anthocyanin gene regulation in plants. However, the mechanisms that control the coordinated expression of structural genes by the MBW complex have long been elusive, in part, due to the following reasons: (1) the promoters of the structural genes in the pathway lack obvious conservation of *cis*-elements. For example, the upstream regulatory regions of the *A1* (*DFR*) and *Bz1* (*UFGT*) promoters contain multiple MYB binding sites, but they are not identical. In addition, the *Bz1* promoter contains an E-box that is commonly recognized by bHLH TFs, whereas no such sequence is present on the *A1* promoter; (2) The bHLH TF, R, despite having a canonical bHLH domain and being capable of activating both *A1* and *Bz1* genes, was thought to be unable to bind the promoters in the absence of the interacting MYB factor, C1. Therefore, the role of R in DNA recognition by the MBW complex was unclear. However, a recent study has demonstrated that an extended bHLH domain of R can homodimerize and bind to the G/E-box sequences in promoters. Dimerization of the bHLH domain is controlled by another dimer-interphase, the ACT-domain [38], which acts as a switch that permits distinct configurations of the C1/R regulatory complex to be tethered to different promoters. R is recruited to the *A1* promoter only through interaction with C1. Activation of the *A1* promoter also requires RIF1, which interacts with a monomeric form of the bHLH domain of R. This monomeric form of the R-bHLH domain is preserved by dimerization of the ACT domain. When dimerization of the ACT domain is disrupted, the bHLH domain is licensed to dimerize and recognize the E-box sequence of the *Bz1* promoter. C1 continues to be essential for this activation as it provides a strong activation domain. RIF1 is not significantly recruited to the *Bz1* promoter and is thus not essential for *Bz1* activation. These findings help clarify the question of how promoters in the anthocyanin pathway lacking obviously conserved elements are coordinately regulated [109].

Micro-RNA and small interfering RNAs (miRNA and siRNA) are important components of a gene regulatory network. Recent studies indicate that they are also involved in the regulation of anthocyanin biosynthesis in plants. In *Arabidopsis*, miRNA828 mediates the cleavage of *Trans-Acting SiRNA Gene 4* (*TAS 4*) transcripts and results in the production of small interfering RNAs (*ta*-siRNA). One such siRNA, *TAS4*-siRNA81(−), targets the MYB TFs, PAP1, PAP2 and MYB113. miRNA828 is also predicted to affect MYB113 expression at an independent target site [7,110] (Fig. 1b). Multiple lines of evidence suggest that a feed-back regulatory loop exists between *PAP1* and *TAS4*: (1) higher accumulation of *PAP1* transcripts and anthocyanin in *ta*-siRNA biogenesis mutants, (2) induction of *TAS*-siRNA81(−) transcripts by physiological concentrations of sucrose and glucose in the PAP1 activation tagged mutant (*pap1-D*), and (3) significant induction of *PAP1* and *TAS*-siRNA81(−) transcripts in phosphate-starved *Arabidopsis* shoots that accumulate high anthocyanin pigments [7,111].

In *Arabidopsis*, anthocyanin mainly accumulates in the junction of rosette and stem. This spatial expression pattern is controlled by microRNA156-targeted SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9 (SPL9), a key regulator of phase change and flowering. Increased *miRNA156* activity represses *SPL9* and induces the expression of key anthocyanin pathway genes and TFs that result in higher anthocyanin synthesis. On the other hand, reduced *miR156* activity promotes higher expression of *SPL9* and results in higher levels of flavonol. TT8 and SPL9 have been shown to bind to the same R2R3 domain of PAP1, and therefore, higher levels of SPL9 in cells destabilize the MBW complex, resulting in a reduction of anthocyanin biosynthesis [112].

Posttranslational regulation of TFs through the ubiquitin/26S proteasome (UPS) system is well documented in plants. In *Arabidopsis*, the loss of 26S proteasome function is accompanied by an increase in anthocyanin accumulation, suggesting a possible role of UPS in regulating the activities of structural genes and/or regulators in flavonoid pathway [113,114]. In *Arabidopsis*, JA-induced anthocyanin accumulation is regulated by Jasmonate ZIM domain (JAZ) proteins and the F-Box

protein, CORONATINE INSENSITIVE 1 (COI1)-based SCF^{COI} complex. The JAZ proteins interact with bHLH TFs (GL3, EGL3, TT8) and R2R3 MYBs (PAP1, PAP2) preventing the formation of an active MBW complex [115]. Upon perception of the JA signal, the JAZ proteins are degraded by the SCF^{COI} complex through 26S UPS, thereby releasing bHLH and MYB factors to form the active MBW complex. The downstream pathway is subsequently triggered, resulting in accumulation of anthocyanin [115] (Fig. 1b). Recently, four functionally redundant bHLH TFs from subgroup IIId, namely bHLH3, bHLH13, bHLH14, bHLH17, have also been shown to interact with JAZ proteins and participate in JA-mediated anthocyanin accumulation in *Arabidopsis*. These bHLH factors act as transcriptional repressors and bind directly to the promoter elements of downstream target genes such as *DFR* in the flavonoid pathway [116].

The stability of the light inducible R2R3 MYB TF, MdMYB1, responsible for apple skin color, has also been shown to be regulated by UPS. The stability of MdMYB1 in dark is regulated by MdCOP1, a photomorphogenesis repressor with E3 ligase activity, through UPS mediated degradation [6]. Recently, it has been demonstrated that the *Arabidopsis* bHLH factors (GL3, EGL3 and TT8), the R2R3 MYBs (PAP1 and PAP2), and the WDR protein (TTG1), which control most of the late biosynthesis genes in the flavonoid pathway, are targeted for proteasomal degradation [8–10]. The HECT E3 ligase, UPL3, mediates the degradation of GL3 and EGL3, whereas the stability of PAP1 and PAP2 is controlled by the ring domain E3 ligase, COP1/SPA (Fig. 1b).

Posttranslational control mechanisms have also been implicated in TIA, nicotine and camalexin biosynthesis in plants. JA-responsive expression of TIA pathway genes is controlled by a cascade of TFs involving CrMYC2 and ORCAs. It was assumed JA-induced accumulation of CrMYC2 activates the expression of *ORCA3*, which in turn, induces the expression of pathway genes, such as *TDC* and *STR*. However, expression studies using the protein synthesis inhibitor, cycloheximide (CHX), showed that the JA-induced expression of these genes is insensitive to CHX. Therefore, *ORCA3* induction by CrMYC2 does not require de novo synthesis of these proteins, but is instead, caused by activation of the pre-existing proteins. Analogous to the regulation of AtMYC2 by AtJAZs, activation of CrMYC2 probably involves JA-induced degradation of JAZ proteins that forms a repressor complex with CrMYC2 in the absence of JA. Isolation and characterization of the JAZ and COI genes from *Catharanthus* will help to elucidate the possible role of the JAZ–COI complex in TIA gene regulation. JA-induced expression of *STR* has been shown to be sensitive to protein kinase inhibitor, suggesting that protein phosphorylation probably plays a role in TIA gene regulation [117]. Whether the pre-existing ORCA proteins are phosphorylated on perception of the JA signal, prior to induction of the pathway gene expression, remains to be clarified. A mitogen-activated protein kinase, CrMPK3, has been isolated from *Catharanthus*. However, its role in posttranslational regulation of the TIA pathway remains to be undefined [118].

Biosynthesis of nicotine is also induced by JA in a COI1-JAZ-dependent manner. Recently, Zhang et al. [119] have shown that tobacco contains multiple *MYC2* genes (*NtMYC2a*, *NtMYC2b*, and *NtMYC2c*) involved in nicotine biosynthesis. The NtJAZ proteins form complexes with NtMYC2s to regulate the JA induction of nicotine biosynthesis [119]. Moreover, a JA-induced phosphorylation cascade has also been shown to play a crucial role in nicotine biosynthesis [87]. Protein phosphorylation is also involved in the regulation of camalexin biosynthesis [70].

The *mediator* complex has been identified as a new component of the transcriptional machinery in plants. Mediator is a large multi-protein complex that is conserved in all eukaryotes [120]. However, it is less well characterized in plants. A mediator complex sub-unit, MEDIATOR 25 (MED 25), also known as PHYTOCHROME AND FLOWERINGTIME 1 (PFT1), has recently been described as a positive regulator of JA signaling in plants. In plants, anthocyanin accumulation is also induced by JA, and MYC2 has been identified as a major regulator of JA signaling. The JA-induced expression of *MYC2* is negatively affected in the *pft* mutant. In addition, the basal expression level of *PAL1*, an early gene in the phenylpropanoid pathway, is lower in the *pft* mutant compared to wild type plants. Consistent with this expression data, *pft* mutants show a decreased anthocyanin accumulation, whereas wild type and *PFT* overexpression lines show strong anthocyanin accumulation [120]. These findings suggest a role of the mediator complex in controlling flavonoid biosynthesis in plants.

6. Concluding remarks

Flavonoid accumulation in plants is under tight spatio-temporal regulation and influenced by a number of biotic and abiotic factors. This is achieved by coordinated transcription of flavonoid pathway genes through transcriptional activators and repressors. However, the molecular mechanism(s) that govern the regulation still remains elusive. Several recent reports have provided insightful information about the regulatory mechanism [47,109]. Many promoters of the coordinately regulated flavonoid pathway genes lack obviously conserved or canonical binding sites for the target TFs. Therefore, identification of potential *cis*-regulatory motifs or modules in the promoters and deciphering their interaction with specific TF complexes will provide useful information on regulation of pathway genes. Such information can potentially be used to engineer metabolic pathways to enhance the accumulation of health promoting compounds in crop plants.

The regulation of alkaloid biosynthesis is relatively complex and still poorly understood. The lack of genetic tools is a major bottle-neck in identifying potential regulators involved in pathway regulation. In *Catharanthus*, regulation of only two TIA pathway genes (*TDC* and *STR*) has been extensively studied. *Cis*-regulatory motifs in the promoter have proven invaluable in the isolation of some of the key regulatory proteins in TIA pathway. Therefore, isolation and characterization of key pathway gene promoters could provide useful hints about TFs involved in pathway regulation, and the *cis*-motifs present in the promoters can be used as a tool to isolate novel factors. Recently, the Medplants Consortium (http://medplants.ncgr.org), Medicinal Plant Genome Resource (MPGR) (http://medicinalplantgenomics.msu.edu) and PhytoMetaSyn (www.phytometasyn.ca) databases have been constructed to advance the research on secondary metabolite biosynthesis and regulation. MPGR provides transcriptome sequences, transcriptome expression, and metabolomic data for 14 medicinal plant species, including *C. roseus*. The available data has already proven useful for the identification of IRIDOID SYNTHASE [121] and a unique cytochrome P450 enzyme, tabersonine/lochnericine 19-hydroxylase [122], involved in MIA biosynthesis in *Catharanthus* [123]. These databases promise to be useful for identifying new regulators in the pathway.

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References

[1] V. De Luca, B. St Pierre, The cell and developmental biology of alkaloid biosynthesis, Trends Plant Sci. 5 (2000) 168–173.
[2] D.A. Nagegowda, Plant volatile terpenoid metabolism: biosynthetic genes, transcriptional regulation and subcellular compartmentation, FEBS Lett. 584 (2010) 2965–2973.
[3] B.S. Winkel, Metabolic channeling in plants, Annu. Rev. Plant Biol. 55 (2004) 85–107.
[4] E. Butelli, L. Titta, M. Giorgio, H.P. Mock, A. Matros, S. Peterek, E.G. Schijlen, R.D. Hall, A.G. Bovy, J. Luo, C. Martin, Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors, Nat. Biotechnol. 26 (2008) 1301–1308.
[5] C.Q. Yang, X. Fang, X.M. Wu, Y.B. Mao, L.J. Wang, X.Y. Chen, Transcriptional regulation of plant secondary metabolism, J. Integr. Plant Biol. 54 (2012) 703–712.

[6] Y.Y. Li, K. Mao, C. Zhao, X.Y. Zhao, H.L. Zhang, H.R. Shu, Y.J. Hao, MdCOP1 ubiquitin E3 ligases interact with MdMYB1 to regulate light-induced anthocyanin biosynthesis and red fruit coloration in apple, Plant Physiol. 160 (2012) 1011–1022.
[7] Q.J. Luo, A. Mittal, F. Jia, C.D. Rock, An autoregulatory feedback loop involving PAP1 and TAS4 in response to sugars in *Arabidopsis*, Plant Mol. Biol. 80 (2012) 117–129.
[8] A. Maier, A. Schrader, L. Kokkelink, C. Falke, B. Welter, E. Iniesto, V. Rubio, J.F. Uhrig, M. Hulskamp, U. Hoecker, Light and the E3 ubiquitin ligase COP1/SPA control the protein stability of the MYB transcription factors PAP1 and PAP2 involved in anthocyanin accumulation in *Arabidopsis*, Plant J. 74 (2013) 638–651.
[9] B. Patra, S. Pattanaik, L. Yuan, Proteolytic degradation of the flavonoid regulators, TRANSPARENT TESTA8 and TRANSPARENT TESTA GLABRA1, in *Arabidopsis* is mediated by the ubiquitin/26Sproteasome system, Plant Signal. Behav. 8 (2013).
[10] B. Patra, S. Pattanaik, L. Yuan, Ubiquitin protein ligase 3 mediates the proteasomal degradation of GLABROUS 3 and ENHANCER OF GLABROUS 3, regulators of trichome development and flavonoid biosynthesis in *Arabidopsis*, Plant J. 74 (2013) 435–447.
[11] N.A. Ramsay, B.J. Glover, MYB-bHLH-WD40 protein complex and the evolution of cellular diversity, Trends Plant Sci. 10 (2005) 63–70.
[12] I. Hichri, F. Barrieu, J. Bogs, C. Kappel, S. Delrot, V. Lauvergeat, Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway, J. Exp. Bot. 62 (2011) 2465–2483.
[13] K. Petroni, C. Tonelli, Recent advances on the regulation of anthocyanin synthesis in reproductive organs, Plant Sci. 181 (2011) 219–229.
[14] E. Grotewold, The genetics and biochemistry of floral pigments, Annu. Rev. Plant Biol. 57 (2006) 761–780.
[15] R. Stracke, H. Ishihara, G. Huep, A. Barsch, F. Mehrtens, K. Niehaus, B. Weisshaar, Differential regulation of closely related R2R3-MYB transcription factors controls flavonol accumulation in different parts of the *Arabidopsis thaliana* seedling, Plant J. 50 (2007) 660–677.
[16] A. Gonzalez, M. Zhao, J.M. Leavitt, A.M. Lloyd, Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/Myb transcriptional complex in *Arabidopsis* seedlings, Plant J. 53 (2008) 814–827.
[17] C. Dubos, J. Le Gourrierec, A. Baudry, G. Huep, E. Lanet, I. Debeaujon, J.M. Routaboul, A. Alboresi, B. Weisshaar, L. Lepiniec, MYBL2 is a new regulator of flavonoid biosynthesis in *Arabidopsis thaliana*, Plant J. 55 (2008) 940–953.
[18] K. Matsui, Y. Umemura, M. Ohme-Takagi, AtMYBL2, a protein with a single MYB domain, acts as a negative regulator of anthocyanin biosynthesis in *Arabidopsis*, Plant J. 55 (2008) 954–967.
[19] H.F. Zhu, K. Fitzsimmons, A. Khandelwal, R.G. Kranz, CPC, a single-repeat R3 MYB, is a negative regulator of anthocyanin biosynthesis in *Arabidopsis*, Mol. Plant 2 (2009) 790–802.
[20] A. Aharoni, C.H. De Vos, M. Wein, Z. Sun, R. Greco, A. Kroon, J.N. Mol, A.P. O'Connell, The strawberry FaMYB1 transcription factor suppresses anthocyanin and flavonol accumulation in transgenic tobacco, Plant J. 28 (2001) 319–332.
[21] E. Loreti, G. Povero, G. Novi, C. Solfanelli, A. Alpi, P. Perata, Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyanin biosynthetic genes in *Arabidopsis*, New Phytol. 179 (2008) 1004–1016.
[22] S. Teng, J. Keurentjes, L. Bentsink, M. Koornneef, S. Smeekens, Sucrose-specific induction of anthocyanin biosynthesis in *Arabidopsis* requires the MYB75/PAP1 gene, Plant Physiol. 139 (2005) 1840–1852.
[23] T. Morishita, Y. Kojima, T. Maruta, A. Nishizawa-Yokoi, Y. Yabuta, S. Shigeoka, *Arabidopsis* NAC transcription factor, ANAC078, regulates flavonoid biosynthesis under high-light, Plant Cell Physiol. 50 (2009) 2210–2222.
[24] C. Spelt, F. Quattrocchio, J.N. Mol, R. Koes, anthocyanin1 of petunia encodes a basic helix–loop–helix protein that directly activates transcription of structural anthocyanin genes, Plant Cell 12 (2000) 1619–1632.
[25] F. Quattrocchio, J. Wing, K. van der Woude, E. Souer, N. de Vetten, J. Mol, R. Koes, Molecular analysis of the anthocyanin2 gene of petunia and its role in the evolution of flower color, Plant Cell 11 (1999) 1433–1444.
[26] C. Spelt, F. Quattrocchio, J. Mol, R. Koes, ANTHOCYANIN1 of petunia controls pigment synthesis, vacuolar pH, and seed coat development by genetically distinct mechanisms, Plant Cell 14 (2002) 2121–2135.
[27] N. de Vetten, F. Quattrocchio, J. Mol, R. Koes, The an11 locus controlling flower pigmentation in petunia encodes a novel WD-repeat protein conserved in yeast, plants, and animals, Genes Dev. 11 (1997) 1422–1434.
[28] F. Quattrocchio, J.F. Wing, K. van der Woude, J.N. Mol, R. Koes, Analysis of bHLH and MYB domain proteins: species-specific regulatory differences are caused by divergent evolution of target anthocyanin genes, Plant J. 13 (1998) 475–488.
[29] N.W. Albert, D.H. Lewis, H. Zhang, K.E. Schwinn, P.E. Jameson, K.M. Davies, Members of an R2R3-MYB transcription factor family in Petunia are developmentally and environmentally regulated to control complex floral and vegetative pigmentation patterning, Plant J. 65 (2011) 771–784.
[30] Y. Bai, S. Pattanaik, B. Patra, J.R. Werkman, C.H. Xie, L. Yuan, Flavonoid-related basic helix–loop–helix regulators, NtAn1a and NtAn1b, of tobacco have originated from two ancestors and are functionally active, Planta 234 (2011) 363–375.
[31] S. Pattanaik, Q. Kong, D. Zaitlin, J.R. Werkman, C.H. Xie, B. Patra, L. Yuan, Isolation and functional characterization of a floral tissue-specific R2R3 MYB regulator from tobacco, Planta 231 (2010) 1061–1076.
[32] K. Schwinn, J. Venail, Y. Shang, S. Mackay, V. Alm, E. Butelli, R. Oyama, P. Bailey, K. Davies, C. Martin, A small family of MYB-regulatory genes controls floral pigmentation intensity and patterning in the genus Antirrhinum, Plant Cell 18 (2006) 831–851.
[33] C.C. Carey, J.T. Strahle, D.A. Selinger, V.L. Chandler, Mutations in the pale aleurone color1 regulatory gene of the *Zea mays* anthocyanin pathway have distinct phenotypes relative to the functionally similar TRANSPARENT TESTA GLABRA1 gene in *Arabidopsis thaliana*, Plant Cell 16 (2004) 450–464.
[34] S.A. Goff, K.C. Cone, V.L. Chandler, Functional analysis of the transcriptional activator encoded by the maize B gene: evidence for a direct functional interaction between two classes of regulatory proteins, Genes Dev. 6 (1992) 864–875.
[35] K.C. Cone, S.M. Cocciolone, F.A. Burr, B. Burr, Maize anthocyanin regulatory gene pl is a duplicate of c1 that functions in the plant, Plant Cell 5 (1993) 1795–1805.
[36] S.R. Ludwig, S.R. Wessler, Maize R gene family: tissue-specific helix—loop—helix proteins, Cell 62 (1990) 849–851.
[37] E. Grotewold, M.B. Sainz, L. Tagliani, J.M. Hernandez, B. Bowen, V.L. Chandler, 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 (2000) 13579–13584.
[38] A. Feller, J.M. Hernandez, E. Grotewold, An ACT-like domain participates in the dimerization of several plant basic-helix–loop–helix transcription factors, J. Biol. Chem. 281 (2006) 28964–28974.
[39] J.M. Hernandez, A. Feller, K. Morohashi, K. Frame, E. Grotewold, The basic helix loop helix domain of maize R links transcriptional regulation and histone modifications by recruitment of an EMSY-related factor, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 17222–17227.
[40] A.M. Takos, F.W. Jaffe, S.R. Jacob, J. Bogs, S.P. Robinson, A.R. Walker, Light-induced expression of a MYB gene regulates anthocyanin biosynthesis in red apples, Plant Physiol. 142 (2006) 1216–1232.
[41] R.V. Espley, R.P. Hellens, J. Putterill, D.E. Stevenson, S. Kutty-Amma, A.C. Allan, Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10, Plant J. 49 (2007) 414–427.
[42] K. Lin-Wang, K. Bolitho, K. Grafton, A. Kortstee, S. Karunairetnam, T.K. McGhie, R.V. Espley, R.P. Hellens, A.C. Allan, An R2R3 MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in Rosaceae, BMC Plant Biol. 10 (2010) 50.
[43] Y. Ban, C. Honda, Y. Hatsuyama, M. Igarashi, H. Bessho, T. Moriguchi, Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red coloration in apple skin, Plant Cell Physiol. 48 (2007) 958–970.
[44] L. Deluc, F. Barrieu, C. Marchive, V. Lauvergeat, A. Decendit, T. Richard, J.P. Carde, J.M. Merillon, S. Hamdi, Characterization of a grapevine R2R3-MYB transcription factor that regulates the phenylpropanoid pathway, Plant Physiol. 140 (2006) 499–511.
[45] J.T. Matus, M.J. Poupin, P. Canon, E. Bordeu, J.A. Alcalde, P. Arce-Johnson, Isolation of WDR and bHLH genes related to flavonoid synthesis in grapevine (*Vitis vinifera* L.), Plant Mol. Biol. 72 (2010) 607–620.
[46] I. Hichri, S.C. Heppel, J. Pillet, C. Leon, S. Czemmel, S. Delrot, V. Lauvergeat, J. Bogs, The basic helix–loop–helix transcription factor MYC1 is involved in the regulation of the flavonoid biosynthesis pathway in grapevine, Mol. Plant 3 (2010) 509–523.
[47] W. Xu, D. Grain, J. Le Gourrierec, E. Harscoet, A. Berger, V. Jauvion, A. Scagnelli, N. Berger, P. Bidzinski, Z. Kelemen, F. Salsac, A. Baudry, J.M. Routaboul, L. Lepiniec, C. Dubos, Regulation of flavonoid biosynthesis involves an unexpected complex transcriptional regulation of TT8 expression, in *Arabidopsis*, New Phytol. 198 (2013) 59–70.
[48] P.J. Facchini, V. De Luca, Opium poppy and Madagascar periwinkle: model non-model systems to investigate alkaloid biosynthesis in plants, Plant J. 54 (2008) 763–784.
[49] J. Memelink, P. Gantet, Transcription factors involved in terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*, Phytochem. Rev. 6 (2007) 353–362.
[50] R.J. Vakil, A clinical trial of *Rauwolfia serpentina* in essential hypertension, Br. Heart J. 11 (1949) 350–355.
[51] J.F. Holland, C. Scharlau, S. Gailani, M.J. Krant, K.B. Olson, J. Horton, B.I. Shnider, J.J. Lynch, A. Owens, P.P. Carbone, J. Colsky, D. Grob, S.P. Miller, T.C. Hall, Vincristine treatment of advanced cancer: a cooperative study of 392 cases, Cancer Res. 33 (1973) 1258–1264.
[52] J. Roepke, V. Salim, M. Wu, A.M.K. Thamm, J. Murata, K. Ploss, W. Boland, V. De Luca, Vinca drug components accumulate exclusively in leaf exudates of Madagascar periwinkle, Proc. Natl. Acad. Sci. 107 (2010) 15287–15292.
[53] V. Burlat, A. Oudin, M. Courtois, M. Rideau, B. St-Pierre, Co-expression of three MEP pathway genes and geraniol 10-hydroxylase in internal phloem parenchyma of *Catharanthus roseus* implicates multicellular translocation of intermediates during the biosynthesis of monoterpene indole alkaloids and isoprenoid-derived primary metabolites, Plant J. 38 (2004) 131–141.
[54] J. Murata, J. Roepke, H. Gordon, V. De Luca, The leaf epidermome of *Catharanthus roseus* reveals its biochemical specialization, The Plant Cell Online 20 (2008) 524–542.
[55] P. Verma, A. Mathur, A. Srivastava, A. Mathur, Emerging trends in research on spatial and temporal organization of terpenoid indole alkaloid pathway in *Catharanthus roseus*: a literature update, Protoplasma 249 (2012) 255–268.
[56] M. El-Sayed, R. Verpoorte, Catharanthus terpenoid indole alkaloids: biosynthesis and regulation, Phytochem. Rev. 6 (2007) 277–305.
[57] W.S. Glenn, W. Runguphan, S.E. O'Connor, Recent progress in the metabolic engineering of alkaloids in plant systems, Curr. Opin. Biotechnol. 24 (2013) 354–365.
[58] D.-H. Liu, H.-B. Jin, Y.-H. Chen, L.-J. Cui, W.-W. Ren, Y.-F. Gong, K.-X. Tang, Terpenoid indole alkaloids biosynthesis and metabolic engineering in *Catharanthus roseus*, J. Integr. Plant Biol. 49 (2007) 961–974.
[59] J.K. Okamuro, B. Caster, R. Villarroel, M. Van Montagu, K.D. Jofuku, The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in *Arabidopsis*, Proc. Natl. Acad. Sci. 94 (1997) 7076–7081.
[60] T. Nakano, K. Suzuki, T. Fujimura, H. Shinshi, Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice, Plant Physiol. 140 (2006) 411–432.
[61] F.L. Menke, A. Champion, J.W. Kijne, J. Memelink, A novel jasmonate- and elicitor-responsive element in the periwinkle secondary metabolite biosynthetic gene Str interacts with a jasmonate- and elicitor-inducible AP2-domain transcription factor, ORCA2, EMBO J. 18 (1999) 4455–4463.
[62] L. van der Fits, J. Memelink, ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism, Science 289 (2000) 295–297.

[63] T. Eulgem, P.J. Rushton, S. Robatzek, I.E. Somssich, The WRKY superfamily of plant transcription factors, Trends Plant Sci. 5 (2000) 199–206.
[64] L. Chen, Y. Song, S. Li, L. Zhang, C. Zou, D. Yu, The role of WRKY transcription factors in plant abiotic stresses, Biochim. Biophys. Acta Gene Regul. Mech. 1819 (2012) 120–128.
[65] P. Agarwal, M. Reddy, J. Chikara, WRKY: its structure, evolutionary relationship, DNA-binding selectivity, role in stress tolerance and development of plants, Mol. Biol. Rep. 38 (2011) 3883–3896.
[66] D. Ma, G. Pu, C. Lei, L. Ma, H. Wang, Y. Guo, J. Chen, Z. Du, H. Wang, G. Li, H. Ye, B. Liu, Isolation and characterization of AaWRKY1, an *Artemisia annua* transcription factor that regulates the amorpha-4,11-diene synthase gene, a key gene of artemisinin biosynthesis, Plant Cell Physiol. 50 (2009) 2146–2161.
[67] S. Li, P. Zhang, M. Zhang, C. Fu, L. Yu, Functional analysis of a WRKY transcription factor involved in transcriptional activation of the DBAT gene in *Taxus chinensis*, Plant Biol. 15 (2013) 19–26.
[68] N. Kato, E. Dubouzet, Y. Kokabu, S. Yoshida, Y. Taniguchi, J.G. Dubouzet, K. Yazaki, F. Sato, Identification of a WRKY protein as a transcriptional regulator of benzylisoquinoline alkaloid biosynthesis in *Coptis japonica*, Plant Cell Physiol. 48 (2007) 8–18.
[69] Y.-H. Xu, J.-W. Wang, S. Wang, J.-Y. Wang, X.-Y. Chen, Characterization of GaWRKY1, a cotton transcription factor that regulates the sesquiterpene synthase gene (+)-δ-cadinene synthase-a, Plant Physiol. 135 (2004) 507–515.
[70] G. Mao, X. Meng, Y. Liu, Z. Zheng, Z. Chen, S. Zhang, Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in *Arabidopsis*, Plant Cell 23 (2011) 1639–1653.
[71] N. Suttipanta, S. Pattanaik, M. Kulshrestha, B. Patra, S.K. Singh, L. Yuan, The transcription factor CrWRKY1 positively regulates the terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*, Plant Physiol. 157 (2011) 2081–2093.
[72] B. Pauw, F.A. Hilliou, V.S. Martin, G. Chatel, C.J. de Wolf, A. Champion, M. Pre, B. van Duijn, J.W. Kijne, L. van der Fits, J. Memelink, Zinc finger proteins act as transcriptional repressors of alkaloid biosynthesis genes in *Catharanthus roseus*, J. Biol. Chem. 279 (2004) 52940–52948.
[73] Y. Siberil, S. Benhamron, J. Memelink, N. Giglioli-Guivarc'h, M. Thiersault, B. Boisson, P. Doireau, P. Gantet, *Catharanthus roseus* G-box binding factors 1 and 2 act as repressors of strictosidine synthase gene expression in cell cultures, Plant Mol. Biol. 45 (2001) 477–488.
[74] L. van der Fits, H. Zhang, F.L. Menke, M. Deneka, J. Memelink, A *Catharanthus roseus* BPF-1 homologue interacts with an elicitor-responsive region of the secondary metabolite biosynthetic gene Str and is induced by elicitor via a JA-independent signal transduction pathway, Plant Mol. Biol. 44 (2000) 675–685.
[75] P.B.F. Ouwerkerk, T.O. Trimborn, F. Hilliou, J. Memelink, Nuclear factors GT-1 and 3AF1 interact with multiple sequences within the promoter of the Tdc gene from Madagascar periwinkle: GT-1 is involved in UV light-induced expression, Mol. Gen. Genet. MGG 261 (1999) 610–622.
[76] N. Suttipanta, S. Pattanaik, S. Gunjan, C.H. Xie, J. Littleton, L. Yuan, Promoter analysis of the *Catharanthus roseus* geraniol 10-hydroxylase gene involved in terpenoid indole alkaloid biosynthesis, Biochim. Biophys. Acta Gene Struct. Expr. 1769 (2007) 139–148.
[77] R.J. Aerts, V. De Luca, Phytochrome is involved in the light-regulation of vindoline biosynthesis in *Catharanthus*, Plant Physiol. 100 (1992) 1029–1032.
[78] Q. Wang, F. Yuan, Q. Pan, M. Li, G. Wang, J. Zhao, K. Tang, Isolation and functional analysis of the *Catharanthus roseus* deacetylvindoline-4-*O*-acetyltransferase gene promoter, Plant Cell Rep. 29 (2010) 185–192.
[79] A. Makhzoum, G. Petit-Paly, B. St. Pierre, M. Bernards, Functional analysis of the DAT gene promoter using transient *Catharanthus roseus* and stable *Nicotiana tabacum* transformation systems, Plant Cell Rep. 30 (2011) 1173–1182.
[80] D. Vom Endt, M. Soares e Silva, J.W. Kijne, G. Pasquali, J. Memelink, Identification of a bipartite jasmonate-responsive promoter element in the *Catharanthus roseus* ORCA3 transcription factor gene that interacts specifically with AT-Hook DNA-binding proteins, Plant Physiol. 144 (2007) 1680–1689.
[81] H. Zhang, S. Hedhili, G. Montiel, Y. Zhang, G. Chatel, M. Pré, P. Gantet, J. Memelink, The basic helix–loop–helix transcription factor CrMYC2 controls the jasmonate-responsive expression of the ORCA genes that regulate alkaloid biosynthesis in *Catharanthus roseus*, Plant J. 67 (2011) 61–71.
[82] Z. Yang, B. Patra, R. Li, S. Pattanaik, L. Yuan, Promoter analysis reveals cis-regulatory motifs associated with the expression of the WRKY transcription factor CrWRKY1 in *Catharanthus roseus*, Planta (2013), http://dx.doi.org/10.1007/s00425-013-1949-2.
[83] C. Voelckel, T. Krügel, K. Gase, N. Heidrich, N.M. van Dam, R. Winz, I.T. Baldwin, Anti-sense expression of putrescine N-methyltransferase confirms defensive role of nicotine in *Nicotiana sylvestris* against *Manduca sexta*, Chemoecology 11 (2001) 121–126.
[84] R. Dawson, M.L. Solt, Estimated contributions of root and shoot to the nicotine content of the tobacco plant, Plant Physiol. 34 (1959) 656.
[85] T. Shoji, Y. Yamada, T. Hashimoto, Jasmonate induction of putrescine N-methyltransferase genes in the root of *Nicotiana sylvestris*, Plant Cell Physiol. 41 (2000) 831–839.
[86] T. Shoji, M. Kajikawa, T. Hashimoto, Clustered transcription factor genes regulate nicotine biosynthesis in tobacco, Plant Cell 22 (2010) 3390–3409.
[87] K. De Boer, S. Tilleman, L. Pauwels, R. Vanden Bossche, V. De Sutter, R. Vanderhaeghen, P. Hilson, J.D. Hamill, A. Goossens, APETALA2/ETHYLENE RESPONSE FACTOR and basic helix–loop–helix tobacco transcription factors cooperatively mediate jasmonate-elicited nicotine biosynthesis, Plant J. 66 (2011) 1053–1065.
[88] T. Shoji, T. Hashimoto, Tobacco MYC2 regulates jasmonate-inducible nicotine biosynthesis genes directly and by way of the NIC2-locus ERF genes, Plant Cell Physiol. 52 (2011) 1117–1130.
[89] T. Shoji, T. Hashimoto, Recruitment of a duplicated primary metabolism gene into the nicotine biosynthesis regulon in tobacco, Plant J. 67 (2011) 949–959.
[90] Y. Yamada, Y. Kokabu, K. Chaki, T. Yoshimoto, M. Ohgaki, S. Yoshida, N. Kato, T. Koyama, F. Sato, Isoquinoline alkaloid biosynthesis is regulated by a unique bHLH-type transcription factor in *Coptis japonica*, Plant Cell Physiol. 52 (2011) 1131–1141.
[91] N.R. Apuya, J.-H. Park, L. Zhang, M. Ahyow, P. Davidow, J. Van Fleet, J.C. Rarang, M. Hippley, T.W. Johnson, H.-D. Yoo, A. Trieu, S. Krueger, C.-y. Wu, Y.-p. Lu, R.B. Flavell, S.C. Bobzin, Enhancement of alkaloid production in opium and California poppy by transactivation using heterologous regulatory factors, Plant Biotechnol. J. 6 (2008) 160–175.
[92] E. Glawischnig, Camalexin, Phytochemistry 68 (2007) 401–406.
[93] B. Dombrecht, G.P. Xue, S.J. Sprague, J.A. Kirkegaard, J.J. Ross, J.B. Reid, G.P. Fitt, N. Sewelam, P.M. Schenk, J.M. Manners, K. Kazan, MYC2 differentially modulates diverse jasmonate-dependent functions in *Arabidopsis*, Plant Cell 19 (2007) 2225–2245.
[94] A. Skirycz, M. Reichelt, M. Burow, C. Birkemeyer, J. Rolcik, J. Kopka, M.I. Zanor, J. Gershenzon, M. Strnad, J. Szopa, B. Mueller-Roeber, I. Witt, DOF transcription factor AtDof1.1 (OBP2) is part of a regulatory network controlling glucosinolate biosynthesis in *Arabidopsis*, Plant J. 47 (2006) 10–24.
[95] J.L. Celenza, J.A. Quiel, G.A. Smolen, H. Merrikh, A.R. Silvestro, J. Normanly, J. Bender, The *Arabidopsis* ATR1 Myb transcription factor controls indolic glucosinolate homeostasis, Plant Physiol. 137 (2005) 253–262.
[96] H. Saga, T. Ogawa, K. Kai, H. Suzuki, Y. Ogata, N. Sakurai, D. Shibata, D. Ohta, Identification and characterization of ANAC042, a transcription factor family gene involved in the regulation of camalexin biosynthesis in *Arabidopsis*, Mol. Plant Microbe Interact. 25 (2012) 684–696.
[97] J.-L. Qiu, B.K. Fiil, K. Petersen, H.B. Nielsen, C.J. Botanga, S. Thorgrimsen, K. Palma, M.C. Suarez-Rodriguez, S. Sandbech-Clausen, J. Lichota, P. Brodersen, K.D. Grasser, O. Mattsson, J. Glazebrook, J. Mundy, M. Petersen, *Arabidopsis* MAP kinase 4 regulates gene expression through transcription factor release in the nucleus, EMBO J. 27 (2008) 2214–2221.
[98] E. Andreasson, T. Jenkins, P. Brodersen, S. Thorgrimsen, N.H.T. Petersen, S. Zhu, J.-L. Qiu, P. Micheelsen, A. Rocher, M. Petersen, M.-A. Newman, H. Bjorn Nielsen, H. Hirt, I. Somssich, O. Mattsson, J. Mundy, The MAP kinase substrate MKS1 is a regulator of plant defense responses, EMBO J. 24 (2005) 2579–2589.
[99] S. Raina, D. Wankhede, M. Jaggi, P. Singh, S. Jalmi, B. Raghiram, A. Sheikh, A. Sinha, CrMPK3, a mitogen activated protein kinase from *Catharanthus roseus* and its possible role in stress induced biosynthesis of monoterpenoid indole alkaloids, BMC Plant Biol. 12 (2012) 134.
[100] E. Vranová, D. Coman, W. Gruissem, Structure and dynamics of the isoprenoid pathway network, Mol. Plant 5 (2012) 318–333.
[101] S. Wu, M. Schalk, A. Clark, R.B. Miles, R. Coates, J. Chappell, Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants, Nat. Biotechnol. 24 (2006) 1441–1447.
[102] Z.-X. Yu, J.-X. Li, C.-Q. Yang, W.-L. Hu, L.-J. Wang, X.-Y. Chen, The jasmonate-responsive AP2/ERF transcription factors AaERF1 and AaERF2 positively regulate artemisinin biosynthesis in *Artemisia annua* L. Mol. Plant 5 (2012) 353–365.
[103] X. Lu, L. Zhang, F. Zhang, W. Jiang, Q. Shen, L. Zhang, Z. Lv, G. Wang, K. Tang, AaORA, a trichome-specific AP2/ERF transcription factor of *Artemisia annua*, is a positive regulator in the artemisinin biosynthetic pathway and in disease resistance to *Botrytis cinerea*, New Phytol. 198 (2013) 1191–1202.
[104] R.J. Roth, N. Acton, A simple conversion of artemisinic acid into artemisinin, J. Nat. Prod. 52 (1989) 1183–1185.
[105] K. Walker, R. Croteau, Molecular cloning of a 10-deacetylbaccatin III-10-O-acetyl transferase cDNA from taxus and functional expression in *Escherichia coli*, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 583–587.
[106] P. Zhang, S.-T. Li, T.-T. Liu, C.-H. Fu, P.-P. Zhou, C.-F. Zhao, L.-J. Yu, Overexpression of a 10-deacetylbaccatin III-10-O-acetyltransferase gene leads to increased taxol yield in cells of *Taxus chinensis*, Plant Cell Tissue Organ Cult. 106 (2011) 63–70.
[107] Y.-Y. Chen, L.-F. Wang, L.-J. Dai, S.-G. Yang, W.-M. Tian, Characterization of HbEREBP1, a wound-responsive transcription factor gene in laticifers of *Hevea brasiliensis* Muell, Arg. Mol. Biol. Rep. 39 (2012) 3713–3719.
[108] Q. Zhang, J. Zhu, Y. Ni, Y. Cai, Z. Zhang, Expression profiling of HbWRKY1, an ethephon-induced WRKY gene in latex from *Hevea brasiliensis* in responding to wounding and drought, Trees 26 (2012) 587–595.
[109] Q. Kong, S. Pattanaik, A. Feller, J.R. Werkman, C. Chai, Y. Wang, E. Grotewold, L. Yuan, Regulatory switch enforced by basic helix–loop–helix and ACT-domain mediated dimerizations of the maize transcription factor R, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) E2091–E2097.
[110] R. Rajagopalan, H. Vaucheret, J. Trejo, D.P. Bartel, A diverse and evolutionarily fluid set of microRNAs in *Arabidopsis thaliana*, Genes Dev. 20 (2006) 3407–3425.
[111] L.C. Hsieh, S.I. Lin, A.C. Shih, J.W. Chen, W.Y. Lin, C.Y. Tseng, W.H. Li, T.J. Chiou, Uncovering small RNA-mediated responses to phosphate deficiency in *Arabidopsis* by deep sequencing, Plant Physiol. 151 (2009) 2120–2132.
[112] J.Y. Gou, F.F. Felippes, C.J. Liu, D. Weigel, J.W. Wang, Negative regulation of anthocyanin biosynthesis in *Arabidopsis* by a miR156-targeted SPL transcription factor, Plant Cell 23 (2011) 1512–1522.
[113] J. Smalle, J. Kurepa, P. Yang, E. Babiychuk, S. Kushnir, A. Durski, R.D. Vierstra, Cytokinin growth responses in *Arabidopsis* involve the 26S proteasome subunit RPN12, Plant Cell 14 (2002) 17–32.
[114] A.S. Fatimababy, Y.L. Lin, R. Usharani, R. Radjacommare, H.T. Wang, H.L. Tsai, Y. Lee, H. Fu, Cross-species divergence of the major recognition pathways of ubiquitylated substrates for ubiquitin/26S proteasome-mediated proteolysis, FEBS J. 277 (2010) 796–816.
[115] T. Qi, S. Song, Q. Ren, D. Wu, H. Huang, Y. Chen, M. Fan, W. Peng, C. Ren, D. Xie, The Jasmonate-ZIM-domain proteins interact with the WD-Repeat/bHLH/MYB complexes to regulate Jasmonate-mediated anthocyanin accumulation and trichome initiation in *Arabidopsis thaliana*, Plant Cell 23 (2011) 1795–1814.

[116] S. Song, T. Qi, M. Fan, X. Zhang, H. Gao, H. Huang, D. Wu, H. Guo, D. Xie, The bHLH subgroup IIId factors negatively regulate jasmonate-mediated plant defense and development, PLoS Genet. 9 (2013) e1003653.
[117] F.L. Menke, S. Parchmann, M.J. Mueller, J.W. Kijne, J. Memelink, Involvement of the octadecanoid pathway and protein phosphorylation in fungal elicitor-induced expression of terpenoid indole alkaloid biosynthetic genes in *Catharanthus roseus*, Plant Physiol. 119 (1999) 1289–1296.
[118] S.K. Raina, D.P. Wankhede, M. Jaggi, P. Singh, S.K. Jalmi, B. Raghuram, A.H. Sheikh, A.K. Sinha, CrMPK3, a mitogen activated protein kinase from *Catharanthus roseus* and its possible role in stress induced biosynthesis of monoterpenoid indole alkaloids, BMC Plant Biol. 12 (2012) 134.
[119] H.-B. Zhang, M.T. Bokowiec, P.J. Rushton, S.-C. Han, M.P. Timko, Tobacco transcription factors NtMYC2a and NtMYC2b form nuclear complexes with the NtJAZ1 repressor and regulate multiple jasmonate-inducible steps in nicotine biosynthesis, Mol. Plant 5 (2012) 73–84.
[120] B.N. Kidd, C.I. Edgar, K.K. Kumar, E.A. Aitken, P.M. Schenk, J.M. Manners, K. Kazan, The mediator complex subunit PFT1 is a key regulator of jasmonate-dependent defense in *Arabidopsis*, Plant Cell 21 (2009) 2237–2252.
[121] F. Geu-Flores, N.H. Sherden, V. Courdavault, V. Burlat, W.S. Glenn, C. Wu, E. Nims, Y. Cui, S.E. O'Connor, An alternative route to cyclic terpenes by reductive cyclization in iridoid biosynthesis, Nature 492 (2012) 138–142.
[122] L.A. Giddings, D.K. Liscombe, J.P. Hamilton, K.L. Childs, D. DellaPenna, C.R. Buell, S.E. O'Connor, A stereoselective hydroxylation step of alkaloid biosynthesis by a unique cytochrome P450 in *Catharanthus roseus*, J. Biol. Chem. 286 (2011) 16751–16757.
[123] E. Gongora-Castillo, K.L. Childs, G. Fedewa, J.P. Hamilton, D.K. Liscombe, M. Magallanes-Lundback, K.K. Mandadi, E. Nims, W. Runguphan, B. Vaillancourt, M. Varbanova-Herde, D. Dellapenna, T.D. McKnight, S. O'Connor, C.R. Buell, Development of transcriptomic resources for interrogating the biosynthesis of monoterpene indole alkaloids in medicinal plant species, PLoS One 7 (2012) e52506.