Transcription factors: tools to engineer the production of pharmacologically active plant metabolites

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Plants produce a variety of secondary metabolites, some of which are used as pharmaceuticals or are health promoting as food components. Recent genetic studies on the flavonoid biosynthetic pathway show that transcription factors are efficient new molecular tools for plant metabolic engineering to increase the production of valuable compounds. The use of specific transcription factors would avoid the time-consuming step of acquiring knowledge about all enzymatic steps of a poorly characterized biosynthetic pathway. Although genetic approaches are difficult for most plant species, promoter studies of single-pathway genes and T-DNA activation tagging are feasible alternative approaches for isolating transcription factors, as illustrated for terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*.

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Transcription factors are sequence-specific DNA-binding proteins that interact with the promoter regions of target genes and modulate the rate of initiation of mRNA synthesis by RNA polymerase II. They control the coordinated expression of genes necessary for normal development and functional physiology of eukaryotes. Several transcription factors are specifically involved in the regulation of metabolism.

Because plants are not mobile, they have evolved a large degree of physiological plasticity to adapt to fluctuating external conditions. In addition, many plant species depend on animals for sexual reproduction and seed dispersal. Among the adaptive responses of plants to external cues is the ability to synthesize a large variety of chemical compounds, called secondary metabolites. Plants use these compounds, which belong to different chemical families such as alkaloids and flavonoids, among others to protect themselves against microbial attack, herbivores and UV irradiation. Secondary metabolites also have key roles in attracting flower pollinators (particularly anthocyanin pigments and terpenoid essential oils) and in other beneficial interactions with other organisms.

In plants, protective secondary metabolites are usually induced by external stress signals, whereas other secondary metabolites, such as flower pigments, are expressed in a tissue-specific manner. This regulation is coordinated by specific transcription factors. Several plant secondary metabolites have pharmacological activities in mammals and are used to treat different diseases. The structure of these molecules is often too complex for efficient complete chemical synthesis (Fig. 1) and plants are their only economically viable source, despite the fact that their concentrations are often low.

In this article, we discuss opportunities for using transcription factors to improve the production of pharmaceutically important plant secondary metabolites based on two examples, flavonoids and alkaloids.

Engineering flavonoid biosynthesis with transcription factors

Flavonoids affect human health

Flavonoids belong to the phenylpropanoid group of compounds, which are formed from the amino acid phenylalanine (Fig. 2). Several molecular families, such as anthocyanin pigments, condensed tannins (Fig. 1), antimicrobial phlobaphenes, UV-absorbing flavones, flavonols and sinapate esters, are derived from the flavonoid biosynthesis pathway.

Flavonoids possess antioxidant, anti-inflammatory, anti-allergenic, hepatoprotective, anti-thrombotic, antiviral and anti-carcinogenic activities. For these reasons, several flavonoids are being tested for their inhibitory effects on the replication of HIV and proliferation of tumor cells [1]. Flavonoids are present naturally in several fruits, vegetables and grains, and in some beverages such as tea and red wine. Their pharmacological properties could explain why, when consumed regularly in the human diet, these compounds have pleiotropic health-promoting and disease-preventing activities [2]. For example, condensed tannins in red wine have been associated recently with the inhibition of endothelin-1 synthesis, a key factor in the development of vascular disease and arteriosclerosis [3]. By contrast, condensed tannins have anti-nutritive effects when present in high quantities in ruminant forages [4].

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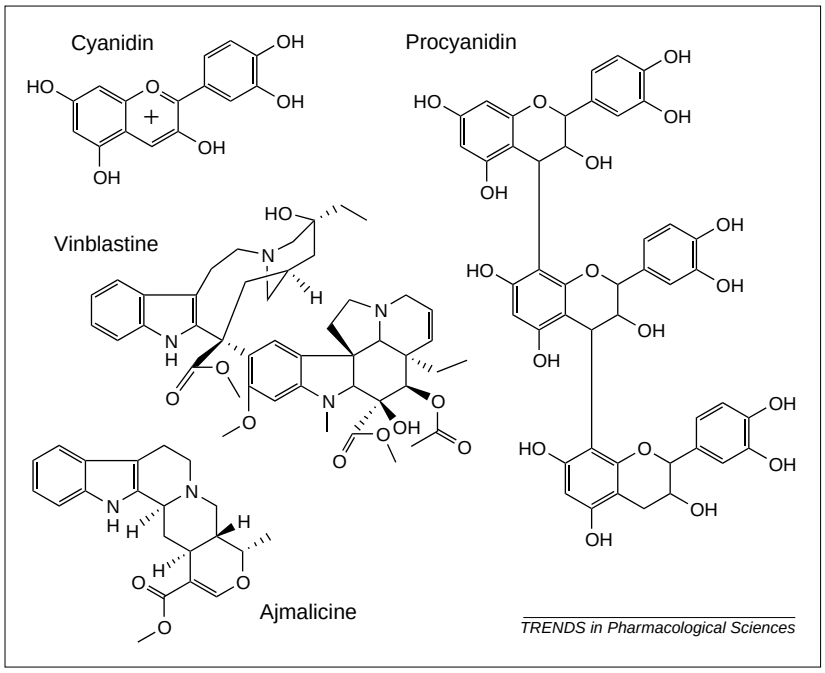

Fig. 1. Chemical structures of some of the bioactive compounds synthesized by plants. Cyanidin (anthocyanin) is an antioxidant, procyanidin (condensed tannin) protects against coronary disease, vinblastine (dimeric terpenoid indole alkaloid) is anti-carcinogenic and ajmalicine (monomeric terpenoid indole alkaloid) is hypotensive.

For these reasons, it could be useful to be able to increase the biosynthesis by plants of specific pharmacologically active flavonoids, either for industrial production or to modify their amounts in human food and animal forage [5].

Anthocyanins: a colorful model for plant metabolic engineering

The anthocyanin branch of the flavonoid biosynthetic pathway has been extensively studied in horticultural and cultivated plant species such as petunia, snapdragon and maize, because anthocyanin pigments determine the colors of flowers and maize seed kernels and thus provide a convenient, visible marker of mutant phenotypes. Other branches of the flavonoid pathway were studied in other species including the plant model *Arabidopsis thaliana*. Together, these studies have led to the isolation of a large number of structural genes (that encode enzymes), genes that encode proteins involved in the intracellular transport of flavonoids into different subcellular compartments, and regulatory genes from different plant species [6].

In plants, the organ-specific and tissue-specific regulation of anthocyanin biosynthesis is controlled by specific transcription factors, which are structurally and functionally well conserved between species [7]. It was first shown in maize that anthocyanin biosynthesis is regulated by a combination of two transcription factor species that are encoded by two families of regulatory genes, *R / B* and *C1 / Pl*. The *R / B* family encodes transcription factors that share homology with the basic helix–loop–helix (bHLH) protein encoded by the vertebrate proto-oncogene *c-MYC*, whereas the *C1 / Pl* genes encode proteins that have homology to the proto-oncogene *c-MYB* product (Table 1). R and C1 interact to regulate anthocyanin biosynthesis in the maize kernel [8]. Homologous regulatory genes regulate anthocyanin synthesis in other parts of maize and other plant species.

Transcription factors control metabolic differentiation of plant cells

When *R* and *C1* are expressed ectopically in unpigmented maize cells cultured *in vitro*, they induce metabolic differentiation that leads to the biosynthesis and accumulation of anthocyanins [9,10]. This correlates with the coordinate expression of the genes that encode enzymes necessary for the transformation of coumaroyl-coA into anthocyanins, the gene that encodes a glutatione *S*-transferase (GST) that is probably required for the vacuolar uptake of the produced pigments [11], and a multidrug-resistant protein transporter that is likely to pump gluthathione-conjugated anthocyanins into the vacuole (Fig. 2) [12]. Cytological studies of the transgenic cells showed the differentiation of both multilamellar bodies within the cytosol and of anthocyanoplasts, which are small vesicles in which anthocyanins accumulate before fusion with the vacuole [9]. Expression-profiling experiments revealed 400 differentially regulated mRNAs, several of which were not known to be regulated by R and C1, such as the genes encoding histone 2B, ribosomal protein L41, α-4 tubulin and MADS box DNA binding domain transcription factors [10]. It was hypothesized that the differential regulation of these latter genes might be associated with the changes in the ultrastructural organization of cells that overexpress R and C1.

These data show that ectopic expression of specific transcription factors can redirect the metabolic differentiation of plant cells by acting simultaneously and coordinately on different events, including the regulation of the expression of genes that encode biosynthetic enzymes and proteins necessary for metabolite storage and differentiation of appropriate subcellular compartments.

Different transcription factors control specific metabolic branches

Similar experiments were done with the regulatory gene *P* in maize [9,10]. *P* encodes a MYB-like transcription factor that is closely related to C1 but can activate the biosynthesis of insecticidal cell-wall-associated flavan-4-ol polymers (phlobaphenes) in maize floral organs without a MYC partner. Ectopic expression of *P* in maize cells shows that P regulates several genes, some of which are also regulated by R and C1 (e.g. the subset of flavonoid genes common to anthocyanin and phlobaphene synthesis), whereas others are specific

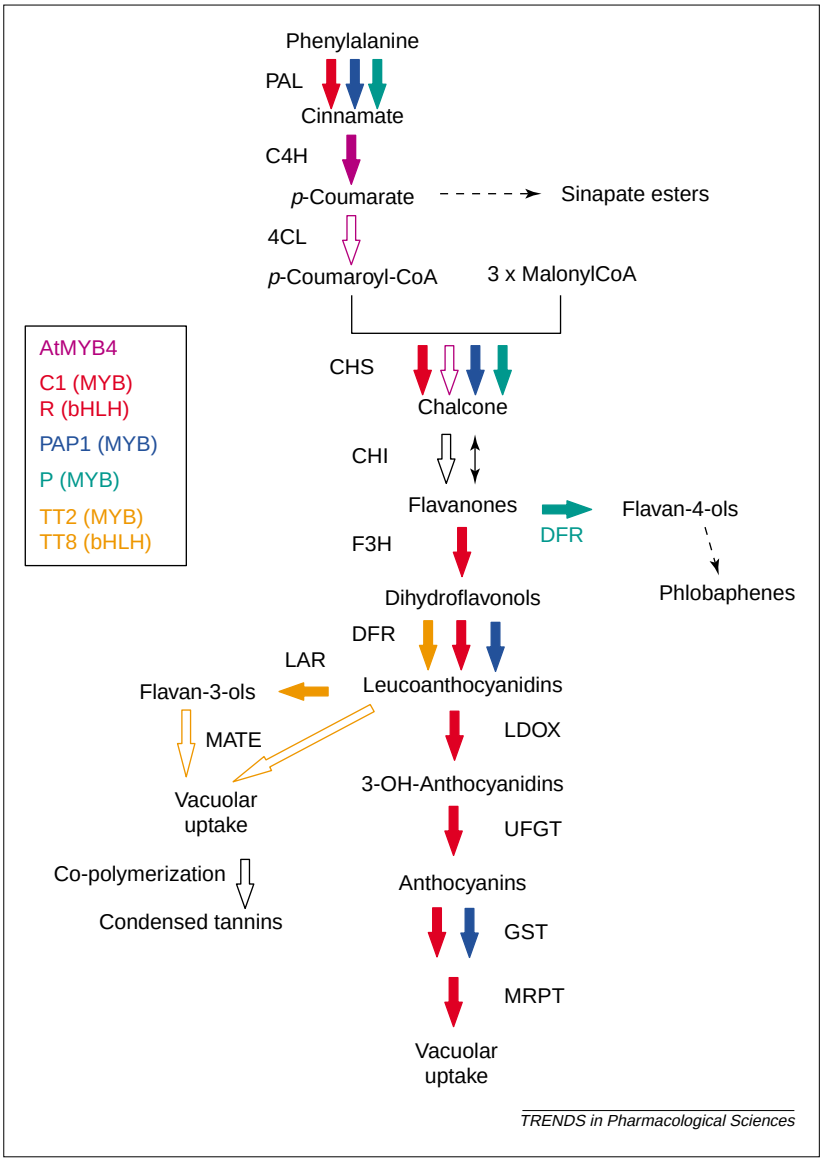

Fig. 2. Regulation of flavonoid biosynthesis by transcription factors. Solid arrows indicate a single enzymatic step and dashed arrows indicate multiple enzymatic steps. Arrow colors match the color of the transcription factor(s) (shown in box on left) that regulate(s) the enzyme-encoding gene. Unfilled arrows indicate that regulation by the corresponding transcription factor(s) is not yet fully demonstrated. The double-headed arrow indicates that the chalcone isomerase (CHI) reaction can occur spontaneously and reversibly without enzymatic intervention. Transcription factors C1, R and P are from maize. MYB4, PAP1, TT2 and TT8 are from *Arabidopsis thaliana*. Abbreviations: At, *Arabidopsis thaliana*; bHLH, basic helix–loop–helix; C4H, cinnamate-4-hydroxylase; CHS, chalcone synthase, 4CL, coumaroyl:CoA-ligase; DFR, dihydroflavonol 4-reductase; F3H, flavanone 3-hydroxylase; GST, glutathione *S*-transferase; LDOX, leucoanthocyanidin dioxygenase; LAR, leucoanthocyanidin reductase; MATE, multidrug and toxin compound extrusion; MRPT multidrug-resistant protein type; PAL, phenylalanine ammonia-lyase; PAP1, production of anthocyanin pigment 1; TT, transparent testa; UFGT, UDPG-flavonoid glucosyl transferase.

for P (Fig. 2). Transgenic *P* cells synthesize flavan-4-ols and are characterized by small, green, autofluorescent vesicles that are secreted to the cell wall where brown compounds accumulate. These data show that different transcription factors can be used to control the production of specific metabolite classes. It is likely that this new toolbox for metabolic engineering can be extended further by isolating transcription factors that regulate other branches of the pathway.

Insight into the regulation of condensed tannin biosynthesis was obtained by analyzing *transparent testa* (*tt*) mutants from *A. thaliana*. These are characterized by decreased brown pigmentation of the seed coat caused by a lack of accumulation of condensed tannins. Several *tt* mutants were found to have functional defects in regulatory genes. *TT8* encodes a bHLH MYC-type and *TT2* a MYB-type transcription factor [13,14]. Analysis of mutant phenotypes and ectopic expression experiments show that TT2 and TT8 cooperate to regulate several genes that encode enzymes involved in the biosynthesis of condensed tannins. In addition, *TT12* encodes a multidrug and toxic-compound-extrusion transporter (Fig. 2) [12–15]. Characterization of the *TT2* and *TT8* regulatory genes opens the way for new strategies to engineer amounts of condensed tannins in crop and forage plants.

Another example is the control of UV-protecting sinapate ester biosynthesis by *AtMYB4* in *A. thaliana* [16]. *AtMYB4* encodes a MYB-type transcription factor that represses expression of the cinnamate-4-hydroxylase (*C4H*) gene and, to a lesser extent, the 4-coumaroyl: CoA-ligase (*4CL*) and chalcone synthase (*CHS*) genes (Fig. 2). The expression of *AtMYB4* is downregulated by exposure to UV light, which leads to derepression of *C4H* expression and results in increased production of sinapate esters. This example emphasizes that, although it is important to be able to transcriptionally activate a biosynthesis pathway, the ability to derepress a pathway by knocking out transcriptional repressors might also be useful.

Identification of new flavonoid regulatory genes via activation tagging

The transcription factors described above were isolated by studying mutant phenotypes that result from gene inactivation. Recent studies emphasize the importance of studying gain-of-function mutants, which can be generated by activation tagging, to identify metabolic regulators. In T-DNA (transferred DNA) activation tagging, a T-DNA insertion element that includes the strong constitutively active 35S promoter from cauliflower mosaic virus (CaMV) at one of its borders is randomly introduced in the plant genome, via *Agrobacterium*-mediated transformation. In each independent transgenic line, the CaMV 35S promoter strongly activates expression of the plant gene to which, by chance, it lies adjacent. By selecting purple plants this method has led to the identification of a new regulatory gene of the anthocyanin pathway in *A. thaliana* [17]. This gene, named *PAP1* (production of anthocyanin pigment 1), encodes a MYB-type transcription factor that is closely related to maize C1 and P. When overexpressed, *PAP1* promotes anthocyanin biosynthesis and accumulation in most parts of the plant and regulates coordinately genes that encode

Table 1. Transcription factors that control secondary metabolism in plants[a]

Plant transcription factor	Metabolite class	Mammalian homolog[b]	Function in mammals	DNA-binding domain
C1	Anthocyanins	c-MYB	Cell cycle	MYB[c]
P	Phlobaphenes			
TT2	Condensed tannins			
PAP1	Anthocyanins			
AtMYB4	Sinapate esters			
CrBPF1	Alkaloids?			
R	Anthocyanins	c-MYC	Cell cycle	bHLH
TT8	Condensed tannins			
CrMYC2	Alkaloids?			
ORCA2	Alkaloids	None		AP2/ERF
ORCA3	Alkaloids			
CrGBF1	Alkaloids?	CREB	Long-term memory, T-cell development, blood pressure	bZIP
CrGBF2	Alkaloids?			

[a]Abbreviations: AP2/ERF, APETALA2/ethylene responsive factor; At, *Arabidopsis thaliana*; bHLH, basic helix–loop–helix; bZIP, basic leucine zipper; Cr, *Catharanthus roseus*; CrBPF1, periwinkle homolog of the MYB-like transcription-factor BPF1; CREB, cAMP response-element binding protein; CrGBF, G-box-binding factor; CrMYC2, MYC-type basic helix–loop–helix transcription factor; ORCA, octadecanoid-responsive *Catharanthus* AP2-domain protein; PAP1, production of anthocyanin pigment 1; TT, transparent testa.
[b]Because there are many mammalian homologs for each class, only one example is given. This corresponds to the first mammalian homolog identified for each plant transcription factor family.
[c]The DNA-binding domains of mammalian MYB proteins contain three repeats. In plants, most MYB proteins contain two repeats although a minority, including CrBPF1, contain a single repeat.

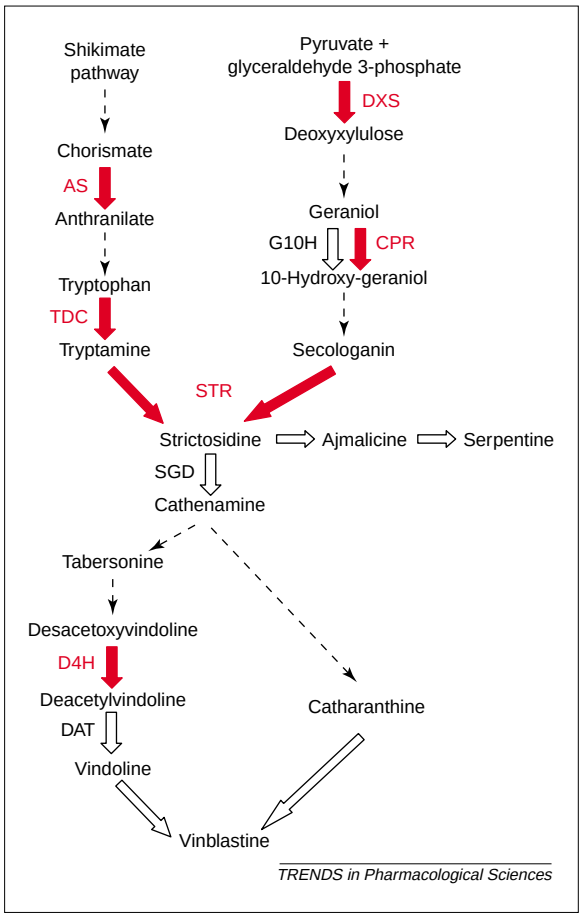

Fig. 3. Regulation of terpenoid indole alkaloid biosynthesis in *Catharanthus roseus* cells by ORCA3. Solid arrows indicate a single enzymatic step and dashed arrows indicate multiple enzymatic steps. Red arrows indicate that the gene encoding the enzyme is regulated by ORCA3. Abbreviations: AS, anthranilate synthase; CPR, cytochrome P450-reductase; DAT, deacetylvindoline acetyltransferase, D4H, desacetoxyvindoline 4-hydroxylase, DXS, D-1-deoxyxylulose 5-phosphate synthase; G10H, geraniol-10-hydroxylase; ORCA3, octadecanoid-responsive *Catharanthus* AP2-domain protein 3; SGD, strictosidine β-D-glucosidase; STR, strictosidine synthase; TDC, tryptophan decarboxylase.

enzymes in the pathway from phenylalanine ammonia-lyase (PAL) to GST (Fig. 2). *PAP1* expression in transgenic tobacco caused similar phenotypes, indicating that the underlying regulatory mechanisms are conserved between species. This is confirmed by the identification of anthocyanin regulatory genes that are homologous to *R* and *C1* in other species [7,18–21]. Nonetheless, studies in which *MYB* and *MYC* regulatory genes from different plant species were expressed in both homologous and heterologous species have identified some variations in their ability to regulate anthocyanin biosynthetic genes [7,18,22].

Engineering alkaloid biosynthesis with transcription factors

Alkaloids: a large family of plant pharmaceutical compounds

Alkaloids are a diverse group of small, heterocyclic, nitrogen-containing molecules (Fig. 1) that are thought to be involved in defending plants against herbivores and pathogens.

Several alkaloids are exploited for their pharmaceutical properties. For example, morphine and codeine are analgesics, vinblastine and taxol are anti-cancer agents, colchicine is a gout suppressant, tubocurarine is a muscle relaxant, sanguinarine is an antibiotic and scopolamine is a sedative. Most of these alkaloids are extracted from plants or are derived either chemically or via biotransformation from natural precursors. There is an increasing demand from the pharmaceutical industry for anti-tumor agents like taxol and vinblastine, and for nicotine for the fabrication of gums and patches designed to help with tobacco weaning. Alkaloid biosynthesis pathways are often more complex than the flavonoid pathway and, at the moment, only a few structural genes from the tropane and benzylisoquinoline alkaloid pathways have been isolated [23,24].

To date, the best-known alkaloid biosynthetic pathway at the gene level leads to the formation of terpenoid indole alkaloids (TIAs) in periwinkle (*Catharanthus roseus*). This plant species produces the monomeric alkaloids serpentine and ajmalicine, which are used as a tranquilizer and to reduce hypertension, respectively. Dimeric alkaloids from periwinkle, vincristine and vinblastine, and their hemisynthetic derivatives, including vinorelbine and

vinflunine, are used extensively in the treatment of many cancers. Dimeric alkaloids are present at very low levels in the plant and are restricted to specific leaf cell types [25]. For this reason the more abundant monomeric precursors, catharanthine and vindoline, are isolated and coupled chemically to form dimeric vinblastine. But even monomeric alkaloid levels are low in plants.

Promoter studies identify regulators of alkaloid biosynthesis

To identify tools to improve alkaloid production, molecular studies on the regulation of the TIA biosynthetic pathway have been carried out [24]. Because of the lack of established genetics and easily visible phenotypes, researchers have focused on the promoter sequences that regulate TIA biosynthetic genes to find transcription factors that control this pathway (Fig. 3). Promoter analysis of the genes that encode strictosidine synthase and tryptophan decarboxylase (*STR* and *TDC*, respectively) reveals that both contain sequences involved in the regulation by stress signals such as UV-irradiation and fungal elicitors [26–29]. Jasmonic acid, a major plant stress hormone, is an essential second messenger for elicitor-induced expression of *STR* and *TDC* [30].

A short *STR* promoter sequence called the JERE (jasmonate and elicitor-responsive element) is responsible for elicitor-responsive and jasmonate-responsive gene expression [31] (Fig. 4). Using the JERE as bait in yeast one-hybrid screening, a cDNA that encodes ORCA2 (octadecanoid-responsive *Catharanthus* AP2-domain protein 2) was isolated. ORCA2 is a transcription factor of the plant-specific AP2/ERF (APETALA2/ethylene-responsive factor) family that is characterized by the presence of an AP2/ERF DNA-binding domain (Table 1) [32]. Transcription of *ORCA2* is rapidly induced by jasmonate and ORCA2 activates *STR* expression by interacting with the JERE [31]. These data indicate that ORCA2 controls the jasmonate-responsive expression of *STR* and, possibly, other TIA biosynthesis genes.

ORCA3, a central regulator of alkaloid biosynthesis

A closely related TIA regulatory gene, called *ORCA3*, was isolated by T-DNA activation tagging in cultured cells from *C. roseus* [33,34]. ORCA3 also binds to the JERE and activates *STR* expression.

ORCA3 expression is also induced by jasmonate [35], which indicates that the functions of ORCA3 and ORCA2 might overlap to regulate jasmonate-responsive expression of alkaloid biosynthetic genes. Ectopic expression of ORCA3 in cultured cells from *C. roseus* increased the expression of the TIA biosynthetic genes *TDC*, *STR*, *CPR* [which encodes a cytochrome P450-reductase that acts with geraniol-10-hydroxylase (G10H) a P450 enzyme] and desacetoxyvindoline 4-hydroxylase. ORCA3 also

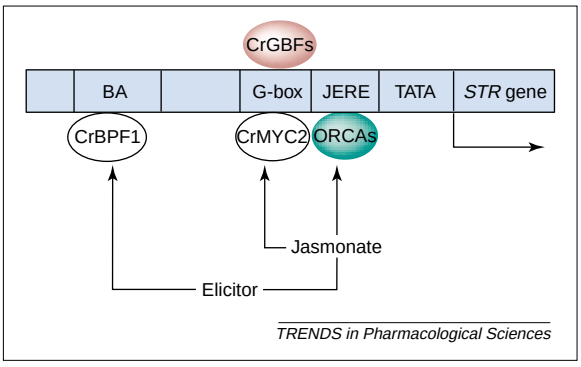

Fig. 4. The promoter region of the strictosidine synthase gene. Transcriptional repressors are red, activators are green, and DNA-binding proteins with unknown transcriptional activity are not colored. Arrows link physiological signals with the transcription factors that regulate gene expression. Abbreviations: BA, an enhancer domain of the *STR* promoter; CrBPF1, periwinkle homolog of the MYB-like transcription-factor BPF1; CrMYC2, MYC-type basic helix–loop–helix transcription factor; G-box, 5′-CACGTG-3′ sequence; JERE, jasmonate and elicitor-responsive element; ORCA, octadecanoid-responsive *Catharanthus* AP2-domain protein; TATA, TATA box.

regulates two genes that encode primary metabolic enzymes (α-subunit of anthranilate synthase and D-1-deoxyxylulose 5-phosphate synthase, respectively) that are involved in the formation of TIA precursors (Fig. 3). This indicates that ORCA3 is a central regulator of TIA biosynthesis that acts pleiotropically on several steps of the TIA pathway and activates the biosynthesis of TIA precursors. Nevertheless, ORCA3 does not regulate the genes encoding G10H and deacetylvindoline acetyltransferase. Transgenic cells that overexpress ORCA3 accumulate significantly more tryptophan and tryptamine. However, because no TIAs are detected, the terpenoid branch of the pathway remains limiting for TIA production. This is confirmed by the increase in TIA production when cells that overexpress ORCA3 are fed with the terpenoid precursor loganin.

Additional transcription factors might control alkaloid biosynthesis

Although *ORCA3* has an important role in regulating TIA biosynthesis, it is not sufficient to regulate the complete pathway. This indicates that other transcription factors are also involved and it would be interesting to know the target genes for, for example, ORCA2.

Using an enhancer domain of the *STR* promoter (Fig. 4) as bait in a yeast one-hybrid screen resulted in the isolation of CrBPF1, a periwinkle homolog of the MYB-like transcription-factor BPF1 from parsley [36]. *CrBPF1* expression is induced by elicitor but not jasmonate, which indicates that elicitor induces *STR* expression in periwinkle cells via jasmonic-acid-dependent and -independent pathways.

In addition, the *STR* promoter contains a promoter element that is conserved in plants, called the G-box (5′-CACGTG-3′; known as the E-box in animal gene promoters) located adjacent to the JERE element

(Fig. 4). The G-box is an active *cis*-regulatory element *in planta* [37]. A yeast one-hybrid screen using the G-box as bait isolated G-box-binding factors (CrGBF) of the basic leucine zipper class and MYC-type bHLH transcription factors (CrMYC) [38]. CrGBF1 and CrGBF2 were shown to repress *STR* expression [39]. They also bind *in vitro* to a G-box-like element in the *TDC* promoter [39], which indicates that CrGBFs could coordinately regulate several TIA biosynthetic genes. Inactivating CrGBF transcription factors might derepress TIA biosynthesis, particularly the terpenoid branch of the pathway. Combined with *ORCA3* overexpression, this would activate TIA biosynthesis. Recombinant CrMYC2 binds to the G-box of *STR in vitro*. *CrMYC2* expression is induced by jasmonate [P. Gantet, unpublished]. The effect of CrMYC2 on *STR* expression has not been tested. Interestingly identification of CrBPF1 (MYB-type) and CrMYC2 indicates that members of these large plant transcription families could be involved in the regulation of both flavonoid and alkaloid biosynthesis pathways. Further studies of these and other transcription factors involved in TIA gene regulation will help to identify additional molecular tools for metabolic engineering of TIA biosynthesis.

Concluding remarks

Because of their pleiotropic action on a wide array of genes involved in metabolic differentiation of plant cells, central transcription factors enable the development of new strategies to engineer complex metabolic pathways and, by increasing the level of pharmaceutically active molecules in plant cells, hold great promise for industrial production. This is of great interest considering that ~25% of contemporary medicines are derived from plants. In addition, plant secondary metabolites represent an enormous chemical diversity with largely unexplored pharmacological activities.

Overexpression of *R* and/or *C1* in heterologous species often results in an increased production of flavonoids. Similarly, ORCA transcription factors might be used to upregulate diverse secondary metabolite pathways that are known to be jasmonate-responsive in other species [24], such as flavonoids, most alkaloid families and naphtoquinones.

Transcription factors that control either a whole biosynthetic pathway or a specialized branch of a pathway that synthesizes natural health-promoting molecules such as flavonoids could also be used to design functional foods or nutraceuticals [40]. An example of this is the design of new vegetables enriched in β-carotene to help prevent vitamin A deficiency. This has been achieved by expressing three enzymes of β-carotene biosynthesis in the endosperm of rice to engineer a golden variety that produces provitamin A in the grain [41].

The use of central transcription factors to modulate plant metabolism avoids the time-consuming step of acquiring knowledge about all the enzymatic steps in a complex metabolic pathway. However, it might require the equally time-consuming step of unraveling complex regulatory networks.

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Calcineurin signaling and neural control of skeletal muscle fiber type and size

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Nerve activity controls muscle contractile function and muscle gene expression. Although excitation–contraction coupling is well characterized, excitation–transcription coupling is still poorly understood. Pharmacological and genetic approaches have been used to dissect the signaling pathways that mediate the effect of nerve activity on muscle fiber type and size. In particular, the role of calcineurin has recently been the subject of intensive investigation and debate. The identification of the transduction pathways involved in neuromuscular signaling has implications for the development of new therapeutic strategies to prevent muscle wasting and loss of muscle power resulting from aging, disuse and neuromuscular disorders.

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Skeletal muscles initially develop in the absence of neural influence; however, their subsequent growth and survival depends on motor innervation. Long-term effects of motor neurons on the muscle fiber phenotype are mediated by two distinct mechanisms: electrical activity, affecting globally the whole muscle fiber; and release of specific neural factors, such as neural agrin and neuregulin, acting only locally at the neuromuscular junction. The crucial role of nerve electrical activity on muscle growth and fiber type profile is clearly illustrated by the dramatic changes induced by motor neuron silencing. Muscle inactivity following spinal cord injury causes muscle atrophy and a slow-to-fast fiber type transformation (Fig. 1). However, electrical stimulation of denervated muscles can prevent muscle atrophy and change the fiber type profile according to the impulse pattern [1]. Identification of the mechanisms that couple electrical signals induced by nerve activity to transcriptional and post-transcriptional changes that affect the muscle phenotype has become the subject of intense research over the past few years. In this article, recent progress in nerve-activity-dependent control of muscle fiber type and size is discussed, focusing on the role of calcineurin signaling *in vivo*.

Nerve-activity-dependent regulation of muscle fiber type and size

Muscle fiber type plasticity

Based on the particular isoform of myosin heavy chain (MyHC) that they express, mammalian skeletal

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