CellPress
OPEN ACCESS

Trends in
Plant Science

Special issue: The power of plant specialised metabolism

Opinion

Enough is enough: feedback control of specialized metabolism

Ying Li [1,2,5,*,@], Erich Grotewold [3] and Natalia Dudareva [1,2,4]

Recent advances in our understanding of plant metabolism have highlighted the significance of specialized metabolites in the regulation of gene expression associated with biosynthetic networks. This opinion article focuses on the molecular mechanisms of small-molecule-mediated feedback regulation at the transcriptional level and its potential modes of action, including metabolite signal perception, the nature of the sensor, and the signaling transduction mechanisms leading to transcriptional and post-transcriptional regulation, based on evidence available from plants and other kingdoms of life. We also discuss the challenges associated with identifying the occurrences, effects, and localization of small molecule–protein interactions. Further understanding of small-molecule-controlled metabolic fluxes will enable rational design of transcriptional regulation systems in metabolic engineering to produce high-value specialized metabolites.

Highlights

Specialized metabolic pathways are highly regulated.

This regulation includes feedback inhibition of the transcriptional machinery by pathway intermediates or products.

Specialized metabolites can be sensed by numerous mechanisms, impacting multiple levels of transcriptional regulation, thus leading to major changes in gene expression.

The regulatory effect of specialized metabolites involves other aspects of gene expression, in addition to transcription.

Central or specialized: does it matter?

Specialized metabolites (see Glossary), often small molecules, display amazing diversity in chemical complexity resulting from distinct biosynthetic networks. To date, little is known about how small molecules control expression of genes that are part of these networks. Due to a general lack of understanding, the control of specialized metabolism has often been deemed as secondary in complexity to the regulation of central metabolism. Recent developments in the field suggest that this is unlikely to be the case. Quite oppositely, sophisticated and creative mechanisms evolved to control specialized metabolism and integrate it with primary metabolism, often involving pathway products in feedback regulation. As our understanding of plant metabolism expands, it is timely and important to note that the distinction between primary and specialized pathways is becoming more blurred in terms that the regulatory mechanisms are highly shared and coordinated. In this opinion article, we focus on the molecular mechanisms of small-molecule-controlled feedback regulation at the transcriptional level, and discuss the possible modes of action, including sensing, signaling, and feedback regulation, as well as the outstanding challenges and future directions. While we focus on feedback regulation triggered by small-molecule specialized metabolites, we also refer to a boarder pool of publications describing sensing of small-molecule primary metabolites, as well as signal perception for processes that are not feedback regulation, given that the basic mechanism can be potentially shared. While plant hormones are small molecules and are often chemically related to specialized metabolites, we are not considering them here because feedback regulatory mechanisms controlling their accumulation are better understood due to their central role in plant development and responses [1,2].

Specialized metabolites and their functions

For successful interaction with the surrounding environment, plants synthesize vast amounts of chemicals referred to as specialized metabolites. By contrast to primary metabolites, which are shared by many plant species and are crucial for fundamental functioning and survival of plants, the specialized compounds are more species-, organ- and tissue-specific [3–7] and play

[1]Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA
[2]Purdue Center for Plant Biology, Purdue University, West Lafayette, IN 47907, USA
[3]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA
[4]Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA
[5]www.purdue.edu/hla/sites/lilab/

*Correspondence:
li2627@purdue.edu (Y. Li).
@Twitter: @LiPurdue (Y. Li).

important roles in above- and below-ground plant defense, attraction of pollinators and seed dispersal, shaping plant–microbiome interactions, plant allelopathy, and protection against biotic and abiotic stresses [8–13]. In addition to acting for plant benefits, these metabolites have much broader impacts by modifying the surrounding ecosystem via plant–plant, plant–insect, and plant–microbe interactions. They also provide powerful nutraceutical ingredients to our diet, and have been extensively used as drugs, pharmaceuticals, insecticides, cosmetics, and food additives [3,14,15].

Most specialized compounds are small, with a molecular weight <1500 Da. They are often synthesized in specific anatomical structures, cell types, and/or subcellular organelles devoted to their production and storage. Specialized metabolites are synthesized using precursors produced by the primary metabolic pathways [16]. Based on their biosynthetic origin, plant specialized compounds are divided into several major classes, including terpenoids, alkaloids, phenylpropanoids, fatty acid derivatives, and amino acid derivatives, with some metabolites containing structural moieties derived from two or more of these classes (e.g., capsaicinoid alkaloids) [16].

While some specialized metabolites accumulate under a variety of spatiotemporal contexts, many are inducible by developmental cues and environmental signals, including biotic or abiotic factors [17]. For example, a subset of specialized metabolites is present in plants at a substantial concentration serving as a primary defense mechanism against herbivores and pathogens, while the induced metabolites, in addition to performing repellent and deterrent functions, could attract natural enemies of the attacking herbivores to protect plants via tritrophic interactions and also inform neighboring plants about the pathogen, thus priming their defenses [11,18]. Since specialized metabolites rely on primary metabolic pathways for precursors, redox power, and energy, their biosynthesis must be carefully coordinated with primary metabolism to minimize the impact on essential cellular functions. How this coordination, particularly in response to developmental and environmental signals, is achieved remains largely unknown. Moreover, the buildup of specialized metabolites can be toxic to plant cells [19,20]. To minimize such detrimental effects, plants efficiently neutralize the potential toxicity of specialized metabolites by chemically modifying them, or physically separating them from other cellular components [20]. Importantly, the overproduction of specialized metabolites could lead to feedback inhibition of their own biosynthetic pathways, including both primary and specialized metabolic pathways, to limit the resources allocated to the production and prevent harmful buildup. Increasingly, studies are demonstrating the existence of crosstalk between metabolites and gene expression, conveying the metabolic information via a feedback regulation of gene transcription [21–24].

Feedback transcriptional regulation: possible mechanisms

One well-known mechanism for how small molecules regulate metabolic flux is through allosteric feedback inhibition (Figure 1), where metabolites bind enzymes within the biosynthetic pathway to reduce their activity and thus the flux through the pathway [25]. On the other hand, the production of specialized metabolites is believed to be regulated primarily at the transcriptional level [22]. Compared to allosteric inhibition of enzymes, which usually suppresses individual steps within the biosynthetic pathways, transcriptional control can affect multiple genes in the metabolic pathways (Figure 1). Such a mechanism allows the timely regulation of multiple biosynthetic steps in the same pathway by turning on as few as a single **transcription factor (TF)**, therefore achieving global coordination and balancing of the metabolic processes [26–28]. For example, in petunia flowers, cellular volatile organic compounds (VOCs) accumulate, caused by a reduction in thickness of the cuticle that acts as a VOC sink/concentrator, triggering feedback inhibition of the expression of genes involved in the VOC biosynthetic network [19]. Highlighting examples of indirect mechanisms by which specialized metabolites influence widespread changes in gene

Glossary

ACT-like domain: structurally related to the aspartokinase, chorismate mutase, and TyrA domain that is known to be involved in allosteric regulation of enzymes by amino acid biosynthesis pathway intermediates; present in ~30% of plant basic helix–loop–helix (bHLH) TFs.

Chromatin-modifying protein (CMP): any protein known to affect chromatin conformation. CMPs include histone-modifying enzymes, adding or removing post-translational modifications (e.g., methylation, acetylation, and ubiquitination) to histones; DNA-modifying enzymes such as DNA methyltransferases and demethylases that alter DNA methylation; and chromatin remodelers that change local chromatin landscapes.

Post-translational modifier (PTM): an enzyme involved in adding or removing post-translational modifications of proteins, including phosphorylation (e.g., kinase and phosphatase), acetylation, SUMOylation, ubiquitination, and methylation.

Specialized metabolites: plant metabolites that are produced in a lineage- and spatiotemporal-specific manner to allow plants to communicate with their surrounding environment, for example: responding to abiotic/biotic stresses, attracting pollinators/seed dispersers, and interacting with neighboring plants. Specialized metabolites are also known as secondary metabolites – by contrast with primary metabolites that are produced by most plant lineages and are essential for plant growth and development – and as natural products in the context of medicinal and industrial applications.

Transcription factor (TF): a regulatory protein that binds DNA in a sequence-specific manner (i.e., by binding specific cis-regulatory elements or CREs) to control the rate of expression of the genes located in close vicinity of the CREs.

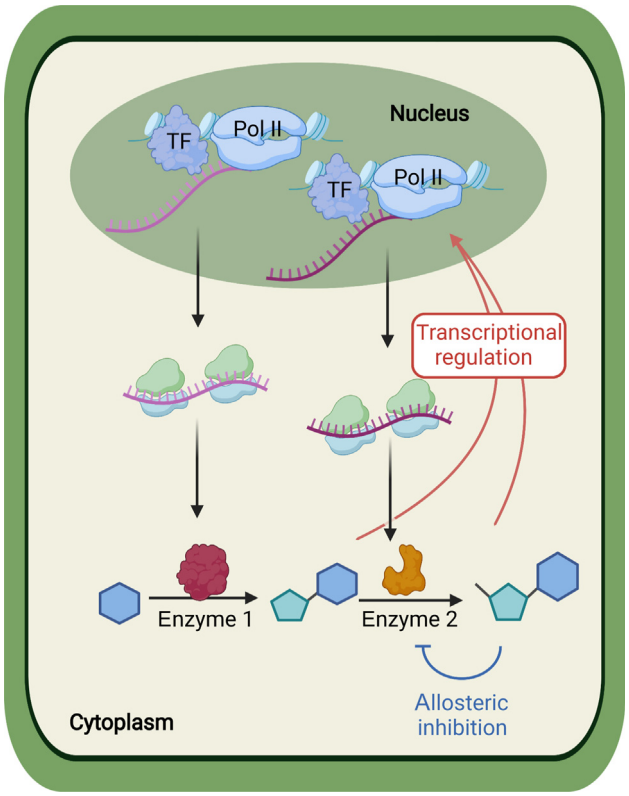

Figure 1. Pathway regulation by small molecules. Transcriptional regulation of a simplified biosynthetic pathway could be triggered by the pathway-produced metabolites (red arrows), acting in parallel with the allosteric inhibition of the biosynthetic enzymes (blue curves). Transcriptional regulation could be positive or negative. Abbreviations: Pol II, RNA polymerase II; TF, transcription factor. Figure created with BioRender.com.

expression: endogenous flavonols have been shown to inhibit auxin transporters [29,30]. Similarly, a reduction in arabidopsis (*Arabidopsis thaliana*) flavonoid levels led to enhanced mRNA accumulation of jasmonic acid biosynthetic and signaling genes upon wounding, suggesting their buffering roles in stress-induced responses [31]. These examples provide evidence that specialized metabolites can impact gene expression directly or indirectly. Another example of gene regulation triggered by small molecules is retrograde signaling. Indeed, multiple small molecules – such as 3′-phosphoadenosine 5′-phosphate (PAP) [32,33], Mg-protoporphyrin (MgProto) [34,35], as well as specialized metabolite precursors such as 2-C-methyl-d-erythritol-2,4-cyclopyrophosphate (MEcPP) [36] – function as retrograde signals to impact nuclear genome expression [34]. Overall, the specific molecular underpinnings of small-molecule-mediated gene regulation remain largely unclear. From a theoretical perspective, small molecules can regulate transcript abundance at the level of *de novo* transcription or post-transcriptional degradation (Figure 2A). For *de novo* transcription, small molecules could affect every step of mRNA biosynthesis, including chromatin remodeling, TF-mediated protein–DNA and protein–protein interactions, as well as function of the general transcriptional machinery (Figure 2A, red arrows I–III).

Small-molecule regulation of transcription factors

In general, transcriptional regulation is controlled largely by TFs, sequence-specific DNA-binding proteins that represent 5–7% of all plant protein-coding genes [37]. TFs recognize conserved *cis*-regulatory elements in the promoters of target genes, thus coordinately regulating transcription of genes within biosynthetic pathways. More often, combinations of TFs collaboratively regulate the

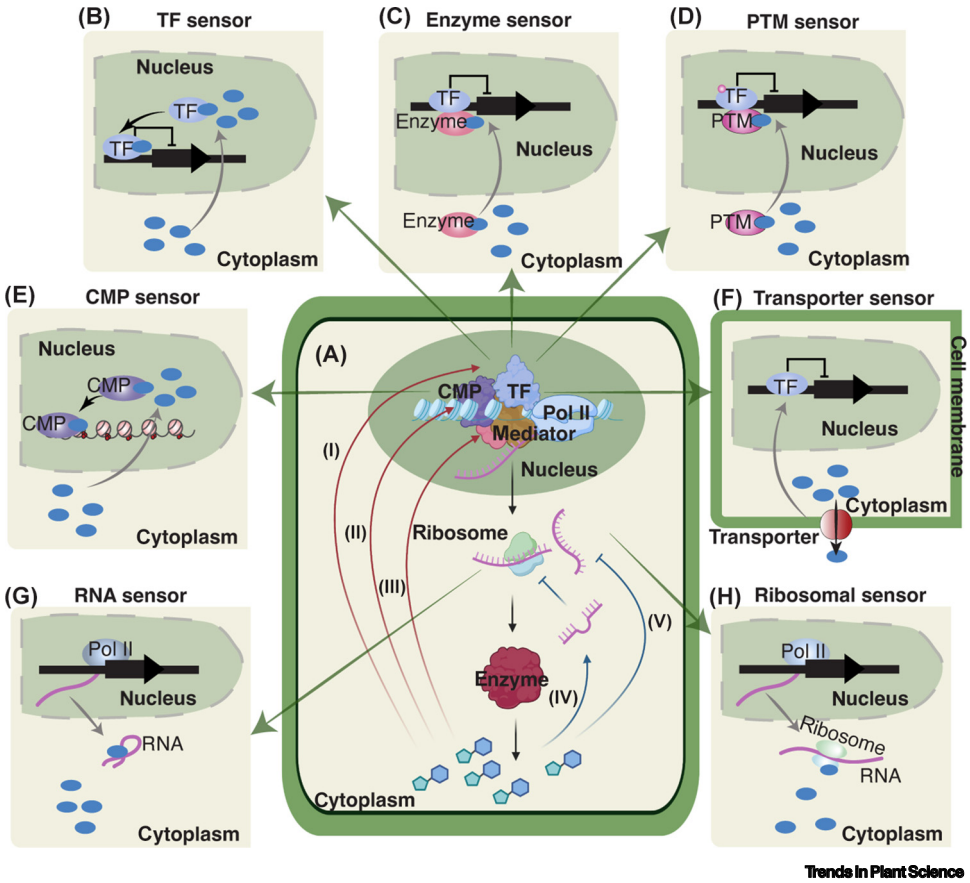

Figure 2. Possible mechanisms involved in the feedback regulation of gene expression triggered by sensing excess amounts of small-molecule specialized metabolites. (A) The metabolites control *de novo* gene transcription (red arrows) through affecting transcription factors (TFs) (I), **chromatin modifying proteins (CMPs)** (II), or general and/or specific transcriptional machinery (III) (Med or Mediator shown as an example). The metabolites could also directly affect mRNAs (blue arrows) through small RNA pathway (IV) or by directly affecting the RNA processing, translation and degradation process (V). Specifically, the metabolites could be sensed by TFs (B), biosynthetic enzymes (C), post-translational modifiers (PTMs) (D), CMPs (E), transporters (F), RNA (G), or ribosomal complex (H). In these scenarios, either the proteins involved in biosynthesis and transport develop the moonlighting function of signal transduction (C,F), or the proteins involved in transcriptional, post-transcriptional, or translational processes evolve the ligand-binding ability to sense the metabolites (B,D,E,H). Alternatively, the sensor could be an RNA molecule rather than protein (G). In most scenarios, the signaling cascade functions across the cytoplasm and nucleus. Abbreviation: Pol II, RNA polymerase II. Figure created partially with BioRender.com.

expression of metabolic pathway genes [22,38], and such combinatorial control increases the specificity and affinity of regulatory complexes for target genes.

Small molecules can modify the activities of TFs to control the expression of a target gene [Figure 2A(I)]. This has been reported in bacteria, yeast, and humans [23,24,39,40]. For example, one of the best characterized bacterial TFs is cAMP receptor protein (CRP), which binds cAMP and regulates the expression of hundreds of genes involved in carbon metabolism [41,42]. In animals, transcriptional regulators BAZ1B, PSIP1, and TSN were shown to sense the level of L-arginine to promote T cell survival [39]. By contrast, similar studies are relatively scarce in plants, especially for specialized metabolites [43–45]. In plants, the green leaf volatiles have been shown to trigger a signaling cascade through the WRKY family TFs to repress gene expression [44] (Table 1).

Table 1. Signaling modules involving small molecules and transcriptional regulators in plants

Plant small molecule	Transcriptional regulator	Class of regulator	Refs
Green leaf volatiles	WRKY6/40	Transcription factor	[44]
Nitrate	NLP7	Transcription factor	[47]
Glucose	Putative TF partners in SCARECROW and MYB families	Transcription factor (through interaction with hexokinase 1)	[45]
Oleoyl-CoA	RAP2.12	Transcription factor	[56]
Monoterpene β-ocimene	HACs and HDA6	Chromatin-modifying protein – histone-modifying enzymes	[58]
Cyclic hydroxamic acids (DIBOA and DIMBOA)	HDAC	Chromatin-modifying protein – histone-modifying enzymes	[59]
Volatile organic compounds (VOCs)	TOPLESS-like proteins (TPLs)	Transcriptional machinery	[43]
Lignin pathway metabolites	Mediator 5a (Med5a)	Transcriptional machinery	[62]
Glucosinolate pathway metabolites	Mediator 5	Transcriptional machinery	[63]

The effect of specialized metabolites on TFs could occur through direct binding to the TFs, or indirectly affecting the activity of a TF. For the former scenario, as TFs contain DNA-binding and transcriptional effector domains, small molecules could physically interact with either one to affect the recognition of the promoter or alter the protein–protein interactions with coregulator partners. In *Escherichia coli*, recent integration of transcriptomics and metabolomics data collected during the growth–starvation–growth switch identified putative metabolite effectors for 71 TFs, and validated direct interactions between five pairs of metabolites and TFs *in vitro* [46]. In plants, the transcriptional regulators that can directly bind and sense internal metabolite levels are largely unknown, and information about small-molecule binding pockets in TFs is scarce. The identification of such relations is further complicated by the noncovalent nature of TF–metabolite interactions. A relevant example from primary metabolism is NIN-like protein 7 (NLP7), the master regulator TF of nitrogen assimilation, which was shown recently to directly bind nitrate in a ligand-binding pocket [47] (Table 1). Thus, we propose that similar mechanisms could be involved in sensing intracellular specialized metabolites (Figure 2B). Indeed, one third of the basic helix–loop–helix (bHLH) family, one of the largest TF families in plants, contain a C-terminal **ACT-like domain** [48–50], which is known in enzymes to be involved in allosteric regulation by pathway intermediates. It raises the question of whether ACT-like domains in the bHLH TFs can also sense the levels of metabolites, thus modulating the ability of the bHLH TF to bind DNA and regulate gene expression [51]. Given the recent developments in protein structure predictions [52], it will be interesting to determine how many other structurally conserved domains are present in plant TFs that could potentially serve as docking sites for small molecules.

Small molecules can affect the activities of TFs in an indirect manner. In this scenario, the metabolic enzymes, for example, could serve as sensors of excessive levels of metabolites and as a result move from the cytoplasm to the nucleus to interact with TFs and regulate gene expression (Figure 2C). Such examples, borrowed from primary metabolism, include arabidopsis hexokinase 1, which senses glucose levels inside a cell and forms a regulatory complex in the nucleus with other proteins including TFs, to alter gene expression [45] (Table 1). Indeed, most glycolytic enzymes in animals moonlight in the nucleus to link metabolism and gene expression [24,53]. In plants, a few studies have provided evidence that specialized metabolism enzymes can be found in the nucleus [54,55]; however, the scope of this nuclear localization of enzymes has yet to be fully explored. Moreover, small molecules also have the potential to control gene expression by interacting with TF **post-translational modifiers (PTMs)** – such as kinases, phosphatases,

transferases, ligases, and proteases – which in turn modulate TF activities (Figure 2D). Finally, small molecules could interact with TF-interacting proteins to modulate TF activities. For example, the central metabolite acyl-coenzyme A (CoA) binds Acyl-CoA-binding protein (ACBP), which releases the interaction between ACBP and TF RAP2.12, thus allowing the TF to move from the plasma membrane to the nucleus [56] (Table 1).

Small-molecule regulation of chromatin regulators

Small molecules can directly interact with chromatin-modifying proteins (Figure 2E) to alter the chromatin landscape of pathway biosynthesis genes to affect their transcription [Figure 2A(II)]. Such a mechanism has been reported in cellular oncogenic transformations, where chromatin-modifying proteins (such as histone acetyltransferases and histone deacetylases) are inhibited by naturally occurring or synthetic small molecules, which have been tested as anticancer drugs [57]. In plants, it was recently shown that monoterpene β-ocimene, released from herbivore-damaged plants, controls expression of defense genes via specific histone acetyltransferases and histone deacetylases [58] (Table 1). Moreover, in plant allelopathy, cyclic hydroxamic acids 2,4-dihydroxy-1,4-benzoxazin-3(4H)-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) were shown to directly bind histone deacetylase to affect histone acetylation and gene expression [59] (Table 1).

Small-molecule regulation of transcriptional machinery

The effects of specialized metabolites on gene regulation can implicate other players in the transcriptional machinery [Figure 2A(III)]. TOPLESS-like proteins (TPLs) are a class of well-studied cosuppressors bridging TFs and chromatin regulators [60]; they possess binding capacity for sesquiterpenes and are involved in sensing VOCs and regulating stress-responsive gene expression [43] (Table 1). Recently, it was shown that TPLs interact with specific Mediator subunits Med10 and Med21, and this interaction was essential for gene repression [61]. Interestingly, the involvement of Mediator complex in sensing specialized metabolites was also proposed for controlling the accumulation of an important plant compound, lignin. In the lignin biosynthetic network, cell-wall-bound or soluble phenylpropanoid metabolites are likely sensed as a signal to maintain phenylpropanoid homeostasis [62] (Table 1). When the metabolic homeostasis is disrupted (e.g., in a lignin-deficient mutant), lignin biosynthesis is repressed, and plants show a pleiotropic stunt growth phenotype, which could be viewed as the 'toxic effect' of metabolic imbalance [62]. This repressive signaling cascade involves the Mediator component Med5a, because mutation in this Mediator component restores the normal growth phenotype of the 'stunt' plants [62]. On a related note, Med5 was involved in activating the expression of *KFB* genes in response to the accumulation of metabolite intermediates in the glucosinolate biosynthetic pathway [63]. The increased activity of KFB proteins then facilitate the degradation of PAL, the committing enzyme of the phenylpropanoid metabolic pathways [63]. In this case, one specialized metabolite (glucosinolate) intermediate controls the activity of another specialized metabolic pathway (phenylpropanoid) using a crosstalk mechanism involving Med5-mediated gene expression as well as post-translational modification of key enzymes [63] (Table 1).

Small-molecule regulation of RNA processing and RNA translation

The effect of small molecules on mRNA levels can function through post-transcriptional regulation of mRNAs, including RNA splicing, RNA translation, and RNA degradation. Apigenin, a flavonoid produced in fruits and vegetables, can directly bind human ribonucleoproteins to affect mRNA splicing and stability, thus modulating the expression levels of a wide range of downstream genes [64]. In arabidopsis, the communication between specialized metabolic pathways (anthocyanin) and small interference RNA (siRNA) pathways has been uncovered [Figure 2A(IV)]. When the anthocyanin pathway was interrupted, mutants in the siRNA pathway (RDR6/SG3/DCL4) led to increased

carbon flux into the flavonoid pathway, suggesting that the siRNA pathways can sense either the end product or intermediates of the anthocyanin pathway and modulate the expression of genes involved in directing flux into the flavonoid pathways [65]. Evidence on the role of another class of small RNA, microRNAs, in regulating plant specialized metabolism has begun to accumulate and was reviewed recently [66]. However, whether specialized metabolites in turn affect miRNA function is barely understood in plants. In cancer biology, natural products such as curcumin (from turmeric root extracts) was shown to exerts its anticancer effects by affecting a range of miRNAs [67]. Additionally, small molecules have been found to interact directly with RNA [Figure 2A(V) and 2G] to affect transcript abundance. Riboswitches, a class of RNA sensors, bind small molecules with a certain degree of specificity, and such interactions affect biosynthesis, processing, translation, and/or degradation of mRNAs [68]. To date, riboswitches have been shown to regulate primary metabolism in bacteria, algae [68], and plants [69]. For example, the 3′ untranslated region (UTR) of the thiamin biosynthetic gene *THIC* contains a riboswitch that senses the level of vitamin B_1 derivative thiamin pyrophosphate (TPP) in arabidopsis [70]. The interaction between TPP and the riboswitch affects the RNA splicing of the *THIC* gene, thus leading to altered RNA stability [68]. This mechanism seems to be highly conserved among plants [68], and has recently been reported for cassava [71] and oil palm [72]. Whether this mechanism is implicated in sensing levels of specialized metabolites is yet to be determined, by uncovering small-molecule–RNA interactions using a combination of *in silico* predictions and biochemical approaches [73]. Finally, metabolites have been shown to control mRNA translation via upstream open reading frame (uORF) and ribosomal stalling as reviewed in [74], including a recent study showing that thermospermine affects the translation of TF-encoding genes *SAC51* and *SACL3* [75]. In this case, it was proposed that thermospermine interacts with ribosomal proteins to alter the translation of target genes (Figure 2H) [75]. Indeed, the concept of ribosomal proteins functioning as metabolite sensors has been considered for several decades in microorganisms and animals [76].

What is being sensed, and by whom?

Thus far, we have discussed different levels of transcriptional as well as post-transcriptional regulation by small molecules, which highlights two key questions: first, what is being sensed, and second, what is the sensor? To monitor the activity of a specialized metabolic pathway, it is possible that the flux through the pathway, or the metabolite levels of the pathway, are being monitored. In the latter scenario, either the end products, or specific intermediates, could be the targets of cellular surveillance mechanisms (Figure 1). Alternatively, the physical or physiological effects caused by the changes in specialized metabolites may trigger feedback regulation. For example, it is possible that cell-wall rigidity – which is influenced mainly by primary metabolites such as cellulose, but is also affected by specialized metabolites – is monitored by mechanosensors that sense cell-wall integrity [77–79] to regulate the flux through lignin biosynthesis pathways.

As for the sensors, due to the daunting diversity of specialized metabolites, the existence of singular-function sensor proteins dedicated for each individual small molecule is rather unlikely. Instead, proteins already involved in biosynthesis or transport of specialized metabolites (Figure 2C and F, respectively) could potentially evolve moonlighting functions as sensors, as seen in glycolytic enzymes [24,53] and nutrient transporters [80]. Under such a mechanism, the specific and efficient ability to bind to a small molecule is dually used by enzymes to sense the level of metabolites and trigger feedback gene regulation, either directly by functioning as part of transcriptional regulatory complexes, or indirectly by participating in the post-translational modification (e.g., acetylation or phosphorylation) of components involved in gene regulation. On a related note, proteins that are already involved in the signaling and (post-)transcriptional regulation of metabolic pathways – such as TFs, chromatin regulators, post-translational modifying enzymes, or ribosomal proteins – could acquire ligand-binding capacities to sense metabolites, hence conveniently linking the

metabolite levels to the signaling regulatory cascade (Figure 2B, D, E, and H). In support of this, indole-3-carbinol, a breakdown product of glucosinolates, was proposed to interact with the auxin receptor [81], providing a potential mechanism for how glucosinolates are sensed to trigger downstream regulation [82,83].

Concluding remarks and future perspectives

From data presented here it is evident that the abundance and diversity of plant specialized metabolites are associated with an equivalent number of opportunities for regulation. There are, however, significant challenges associated with such research, including that mutations affecting the accumulation of specialized metabolites can go largely unnoticed unless plants are grown in the appropriate conditions (which is usually not a growth chamber or greenhouse). Protein–small molecule interactions can also be difficult to demonstrate, because: (i) dissociation constants are often in the micromolar or even millimolar range; (ii) identification of the compounds involved in such interactions is challenging due to their minute quantities in plant cells; (iii) as their biological activities are tightly linked to the structures, developing the knowledge about decorations of the specialized metabolites (e.g., glycosylation, methylation, and acetylation) is crucial yet rather demanding; and (iv) testing such interactions *in vitro* is complicated, as most specialized metabolites are unavailable commercially. Moreover, demonstrating that the small molecule and the potential sensor protein colocalize involves a number of technical challenges, given that the currently available analytical methods to detect small molecules *in situ,* such as imaging mass spectrometry, lack the necessary spatial resolution [84]. Finally, even after the interaction of the small molecule with a protein has been well demonstrated, establishing how it affects protein function, if not an enzyme, can involve significant challenges. Various tools exist for determining the *in vitro* effects of small molecules on modulating protein–protein or protein–DNA interactions; however, determining their *in planta* functions is more difficult. Many of our currently unsuccessful metabolic engineering efforts to produce high yields of specialized metabolites are the result of poor understanding of regulation at the transcriptional level. Understanding the mode of small-molecule action in the regulation of gene expression will provide the foundation for rationally designing transcriptional regulation systems in metabolic engineering with predicted expression strengths (see Outstanding questions).

Outstanding questions

What is the real metabolic cost to plants of making specialized metabolites?

Are there 'sentinel metabolites' that are sensed by the cell, or is feedback a characteristic feature shared by most pathway products/intermediates?

While gene regulation occurs largely within the nucleus, the biosynthesis of specialized metabolites often takes place in the cytoplasm in a highly compartmentalized manner (e.g., in the plastid, or in the endoplasmic reticulum). How does the signaling cascade relay information across multiple cellular compartments? Do metabolites move into the nucleus, and if so, how?

Within a metabolic network, what is the rule governing the choice of targeted metabolic steps to be regulated?

How did the regulation of specialized metabolism evolve?

Acknowledgments

This work was supported by National Science Foundation grants MCB-2123470 to Y.L. and N.D., and IOS-1733633 to E.G.; USDA National Institute of Food and Agriculture Hatch project numbers 1013620 to Y.L. and 177845 to N.D.

Declaration of interests

The authors declare no conflicts of interest.

References

1. Durbak, A. *et al.* (2012) Hormone signaling in plant development. *Curr. Opin. Plant Biol.* 15, 92–96
2. Chung, Y. and Choe, S. (2013) The regulation of brassinosteroid biosynthesis in arabidopsis. *CRC Crit. Rev. Plant Sci.* 32, 396–410
3. O'Connor, S.E. (2015) Engineering of secondary metabolism. *Annu. Rev. Genet.* 49, 71–94
4. Colquhoun, T.A. *et al.* (2010) Petunia floral volatile benzenoid/phenylpropanoid genes are regulated in a similar manner. *Phytochemistry* 71, 158–167
5. Maeda, H. *et al.* (2010) RNAi suppression of arogenate dehydratase 1 reveals that phenylalanine is synthesized predominantly via the arogenate pathway in petunia petals. *Plant Cell* 22, 832–849
6. Verdonk, J.C. *et al.* (2003) Regulation of floral scent production in petunia revealed by targeted metabolomics. *Phytochemistry* 62, 997–1008
7. Wink, M. (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* 64, 3–19
8. Tanaka, Y. *et al.* (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *Plant J.* 54, 733–749
9. Vickers, C.E. *et al.* (2009) A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nat. Chem. Biol.* 5, 283–291
10. Holopainen, J.K. and Gershenzon, J. (2010) Multiple stress factors and the emission of plant VOCs. *Trends Plant Sci.* 15, 176–184
11. Brosset, A. and Blande, J.D. (2022) Volatile-mediated plant–plant interactions: volatile organic compounds as modulators of receiver plant defence, growth, and reproduction. *J. Exp. Bot.* 73, 511–528
12. Jacoby, R.P. *et al.* (2021) Pinpointing secondary metabolites that shape the composition and function of the plant microbiome. *J. Exp. Bot.* 72, 57–69

13. Bennett, R.N. and Wallsgrove, R.M. (1994) Secondary metabolites in plant defence mechanisms. *New Phytol.* 127, 617–633
14. Taroncher, M. *et al.* (2021) Biological activity and toxicity of plant nutraceuticals: an overview. *Curr. Opin. Food Sci.* 42, 113–118
15. Ma, J.K.-C. *et al.* (2005) Plant-derived pharmaceuticals – the road forward. *Trends Plant Sci.* 10, 580–585
16. Wink, M. (2011) *Annual Plant Reviews, Biochemistry of Plant Secondary Metabolism* (2nd edn), 40. John Wiley
17. Mithöfer, A. and Boland, W. (2012) Plant defense against herbivores: chemical aspects. *Annu. Rev. Plant Biol.* 63, 431–450
18. Takabayashi, J. and Shiojiri, K. (2019) Multifunctionality of herbivory-induced plant volatiles in chemical communication in tritrophic interactions. *Curr. Opin. Insect Sci.* 32, 110–117
19. Liao, P. *et al.* (2021) Cuticle thickness affects dynamics of volatile emission from petunia flowers. *Nat. Chem. Biol.* 17, 138–145
20. Sirikantaramas, S. *et al.* (2008) Mechanisms of resistance to self-produced toxic secondary metabolites in plants. *Phytochem. Rev.* 7, 467–477
21. Gaudinier, A. *et al.* (2015) Transcriptional networks governing plant metabolism. *Curr. Plant Biol.* 3–4, 56–64
22. Colinas, M. and Goossens, A. (2018) Combinatorial transcriptional control of plant specialized metabolism. *Trends Plant Sci.* 23, 324–336
23. Donati, S. *et al.* (2018) Crosstalk between transcription and metabolism: how much enzyme is enough for a cell? *Wiley Interdiscip. Rev. Syst. Biol. Med.* 10, e1396
24. Boukouris, A.E. *et al.* (2016) Metabolic enzymes moonlighting in the nucleus: metabolic regulation of gene transcription. *Trends Biochem. Sci.* 41, 712–730
25. Lisi, G.P. and Loria, J.P. (2017) Allostery in enzyme catalysis. *Curr. Opin. Struct. Biol.* 47, 123–130
26. Tzin, V. *et al.* (2013) Tomato fruits expressing a bacterial feedback-insensitive 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase of the shikimate pathway possess enhanced levels of multiple specialized metabolites and upgraded aroma. *J. Exp. Bot.* 64, 4441–4452
27. Xie, Q. *et al.* (2016) Altered metabolite accumulation in tomato fruits by coexpressing a feedback-insensitive AroG and the PhODO1 MYB-type transcription factor. *Plant Biotechnol. J.* 14, 2300–2309
28. Aharoni, A. and Galili, G. (2011) Metabolic engineering of the plant primary–secondary metabolism interface. *Curr. Opin. Biotechnol.* 22, 239–244
29. Brown, D.E. *et al.* (2001) Flavonoids act as negative regulators of auxin transport *in vivo* in arabidopsis. *Plant Physiol.* 126, 524–535
30. Peer, W.A. and Murphy, A.S. (2007) Flavonoids and auxin transport: modulators or regulators? *Trends Plant Sci.* 12, 556–563
31. Pourcel, L. *et al.* (2013) A chemical complementation approach reveals genes and interactions of flavonoids with other pathways. *Plant J.* 74, 383–397
32. Estavillo, G.M. *et al.* (2011) Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in arabidopsis. *Plant Cell* 23, 3992–4012
33. Chan, K.X. *et al.* (2016) Sensing and signaling of oxidative stress in chloroplasts by inactivation of the SAL1 phosphoadenosine phosphatase. *Proc. Natl. Acad. Sci. U. S. A.* 113, E4567–E4576
34. Mielecki, J. *et al.* (2020) Retrograde signaling: understanding the communication between organelles. *Int. J. Mol. Sci.* 21, 6173
35. Woodson, J.D. *et al.* (2011) Heme synthesis by plastid ferrochelatase I regulates nuclear gene expression in plants. *Curr. Biol.* 21, 897–903
36. Wang, J.-Z. *et al.* (2022) Reciprocity between a retrograde signal and a putative metalloprotease reconfigures plastidial metabolic and structural states. *Sci. Adv.* 8, eabo0724
37. Yilmaz, A. *et al.* (2009) GRASSIUS: a platform for comparative regulatory genomics across the grasses. *Plant Physiol.* 149, 171–180
38. Brkljacic, J. and Grotewold, E. (2017) Combinatorial control of plant gene expression. *Biochim. Biophys. Acta Gene Regul. Mech.* 1860, 31–40
39. Geiger, R. *et al.* (2016) L-Arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* 167, 829–842.e13
40. Denison, C. and Kodadek, T. (1998) Small-molecule-based strategies for controlling gene expression. *Chem. Biol.* 5, R129–R145
41. Saier Jr., M.H. (1998) Multiple mechanisms controlling carbon metabolism in bacteria. *Biotechnol. Bioeng.* 58, 170–174
42. Kim, D. *et al.* (2018) Systems assessment of transcriptional regulation on central carbon metabolism by Cra and CRP. *Nucleic Acids Res.* 46, 2901–2917
43. Nagashima, A. *et al.* (2019) Transcriptional regulators involved in responses to volatile organic compounds in plants. *J. Biol. Chem.* 294, 2256–2266
44. Mirabella, R. *et al.* (2015) WRKY40 and WRKY6 act downstream of the green leaf volatile E-2-hexenal in arabidopsis. *Plant J.* 83, 1082–1096
45. Cho, Y.-H. *et al.* (2006) Regulatory functions of nuclear hexokinase1 complex in glucose signaling. *Cell* 127, 579–589
46. Lempp, M. *et al.* (2019) Systematic identification of metabolites controlling gene expression in *E. coli*. *Nat. Commun.* 10, 4463
47. Liu, K.-H. *et al.* (2022) NIN-like protein 7 transcription factor is a plant nitrate sensor. *Science* 377, 1419–1425
48. Grant, G.A. (2006) The ACT domain: a small molecule binding domain and its role as a common regulatory element. *J. Biol. Chem.* 281, 33825–33829
49. Feller, A. *et al.* (2006) An ACT-like domain participates in the dimerization of several plant basic-helix–loop–helix transcription factors. *J. Biol. Chem.* 281, 28964–28974
50. Lee, Y.S. *et al.* (2023) Evolution and diversification of the ACT-like domain associated with plant basic helix–loop–helix transcription factors. *Proc. Natl. Acad. Sci. U. S. A.* 120, e2219469120
51. Lee, Y.S. *et al.* (2021) A hydrophobic residue stabilizes dimers of regulatory ACT-like domains in plant basic helix–loop–helix transcription factors. *J. Biol. Chem.* 296, 100708
52. Jumper, J. *et al.* (2021) Highly accurate protein structure prediction with AlphaFold. *Nature* 596, 583–589
53. Bian, X. *et al.* (2022) Regulation of gene expression by glycolytic and gluconeogenic enzymes. *Trends Cell Biol.* 32, 786–799
54. Stavrinides, A. *et al.* (2015) Unlocking the diversity of alkaloids in *Catharanthus roseus*: nuclear localization suggests metabolic channeling in secondary metabolism. *Chem. Biol.* 22, 336–341
55. Saslowsky, D.E. *et al.* (2005) Nuclear localization of flavonoid enzymes in arabidopsis. *J. Biol. Chem.* 280, 23735–23740
56. Schmidt, R.R. *et al.* (2018) Low-oxygen response is triggered by an ATP-dependent shift in oleoyl-CoA in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 115, E12101–E12110
57. Finley, A. and Copeland, R.A. (2014) Small molecule control of chromatin remodeling. *Chem. Biol.* 21, 1196–1210
58. Onosato, H. *et al.* (2022) Sustained defense response via volatile signaling and its epigenetic transcriptional regulation. *Plant Physiol.* 189, 922–933
59. Venturelli, S. *et al.* (2015) Plants release precursors of histone deacetylase inhibitors to suppress growth of competitors. *Plant Cell* 27, 3175–3189
60. Martin-Arevalillo, R. *et al.* (2017) Structure of the arabidopsis TOPLESS corepressor provides insight into the evolution of transcriptional repression. *Proc. Natl. Acad. Sci. U. S. A.* 114, 8107–8112
61. Leydon, A.R. *et al.* (2021) Repression by the arabidopsis TOPLESS corepressor requires association with the core mediator complex. *eLife* 10, e66739
62. Bonawitz, N.D. *et al.* (2014) Disruption of Mediator rescues the stunted growth of a lignin-deficient arabidopsis mutant. *Nature* 509, 376–380
63. Kim, J.I. *et al.* (2020) Glucosinolate and phenylpropanoid biosynthesis are linked by proteasome-dependent degradation of PAL. *New Phytol.* 225, 154–168
64. Arango, D. *et al.* (2013) Molecular basis for the action of a dietary flavonoid revealed by the comprehensive identification of apigenin human targets. *Proc. Natl. Acad. Sci. U. S. A.* 110, E2153–E2162
65. Jiang, N. *et al.* (2020) Synergy between the anthocyanin and RDR6/SGS3/DCL4 siRNA pathways expose hidden features of arabidopsis carbon metabolism. *Nat. Commun.* 11, 2456
66. Gupta, O.P. *et al.* (2017) Contemporary understanding of miRNA-based regulation of secondary metabolites biosynthesis in plants. *Front. Plant Sci.* 8, 374
67. Mirzaei, H. *et al.* (2018) MicroRNA: a novel target of curcumin in cancer therapy. *J. Cell. Physiol.* 233, 3004–3015

68. Bocobza, S.E. and Aharoni, A. (2014) Small molecules that interact with RNA: riboswitch-based gene control and its involvement in metabolic regulation in plants and algae. *Plant J.* 79, 693–703
69. Bocobza, S.E. *et al.* (2013) Orchestration of thiamin biosynthesis and central metabolism by combined action of the thiamin pyrophosphate riboswitch and the circadian clock in arabidopsis. *Plant Cell* 25, 288–307
70. Wachter, A. *et al.* (2007) Riboswitch control of gene expression in plants by splicing and alternative 3′ end processing of mRNAs. *Plant Cell* 19, 3437–3450
71. Mangel, N. *et al.* (2017) Vitamin B1 diversity and characterization of biosynthesis genes in cassava. *J. Exp. Bot.* 68, 3351–3363
72. Subki, A. *et al.* (2020) Identification and characterisation of thiamine pyrophosphate (TPP) riboswitch in *Elaeis guineensis*. *PLoS One* 15, e0235431
73. Childs-Disney, J.L. *et al.* (2022) Targeting RNA structures with small molecules. *Nat. Rev. Drug Discov.* 21, 736–762
74. van der Horst, S. *et al.* (2020) Metabolite control of translation by conserved peptide uORFs: the ribosome as a metabolite multisensor. *Plant Physiol.* 182, 110–122
75. Yamamoto, M. and Takahashi, T. (2017) Thermospermine enhances translation of SAC51 and SACL1 in arabidopsis. *Plant Signal. Behav.* 12, e1276685
76. Seip, B. and Innis, C.A. (2016) How widespread is metabolite sensing by ribosome-arresting nascent peptides? *J. Mol. Biol.* 428, 2217–2227
77. Bacete, L. *et al.* (2022) THESEUS1 modulates cell wall stiffness and abscisic acid production in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2119258119
78. Zhao, F. and Long, Y. (2022) Mechanosensing, from forces to structures. *Front. Plant Sci.* 13, 1060018
79. Li, C. *et al.* (2016) FERONIA and her pals: functions and mechanisms. *Plant Physiol.* 171, 2379–2392
80. Gojon, A. *et al.* (2011) Nitrate transceptor(s) in plants. *J. Exp. Bot.* 62, 2299–2308
81. Katz, E. *et al.* (2015) The glucosinolate breakdown product indole-3-carbinol acts as an auxin antagonist in roots of *Arabidopsis thaliana*. *Plant J.* 82, 547–555
82. Burow, M. *et al.* (2015) The glucosinolate biosynthetic gene AOP2 mediates feed-back regulation of jasmonic acid signaling in arabidopsis. *Mol. Plant* 8, 1201–1212
83. Katz, E. *et al.* (2020) Diverse allyl glucosinolate catabolites independently influence root growth and development. *Plant Physiol.* 183, 1376–1390
84. Lee, Y.J. *et al.* (2012) Use of mass spectrometry for imaging metabolites in plants. *Plant J.* 70, 81–95