Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Coenzymes and the primary and specialized metabolism interface

Maite Colinas[1] and Teresa B. Fitzpatrick[2]

Abstract

In plants, primary and specialized metabolism have classically been distinguished as *either* essential for growth *or* required for survival in a particular environment. Coenzymes (organic cofactors) are essential for growth but their importance to specialized metabolism is often not considered. In line with the recent proposal of viewing primary and specialized metabolism as an integrated whole rather than segregated lots with a defined interface, we highlight here the importance of collating information on the regulation of coenzyme supply with metabolic demands using examples of vitamin B derived coenzymes. We emphasize that coenzymes can have enormous influence on the outcome of metabolic as well as engineered pathways and should be taken into account in the era of synthetic biology.

Addresses

[1] Department of Natural Product Biosynthesis, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 80, D-07745 Jena, Germany
[2] Department of Botany and Plant Biology, University of Geneva, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland

Corresponding authors: Fitzpatrick, Teresa B (teresa.fitzpatrick@unige.ch); Colinas, Maite (mmartinez@ice.mpg.de)

Current Opinion in Plant Biology 2022, **66**:102170

This review comes from a themed issue on **Physiology and metabolism**

Edited by **Asaph Aharoni** and **Hiroshi A. Maeda**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.pbi.2021.102170

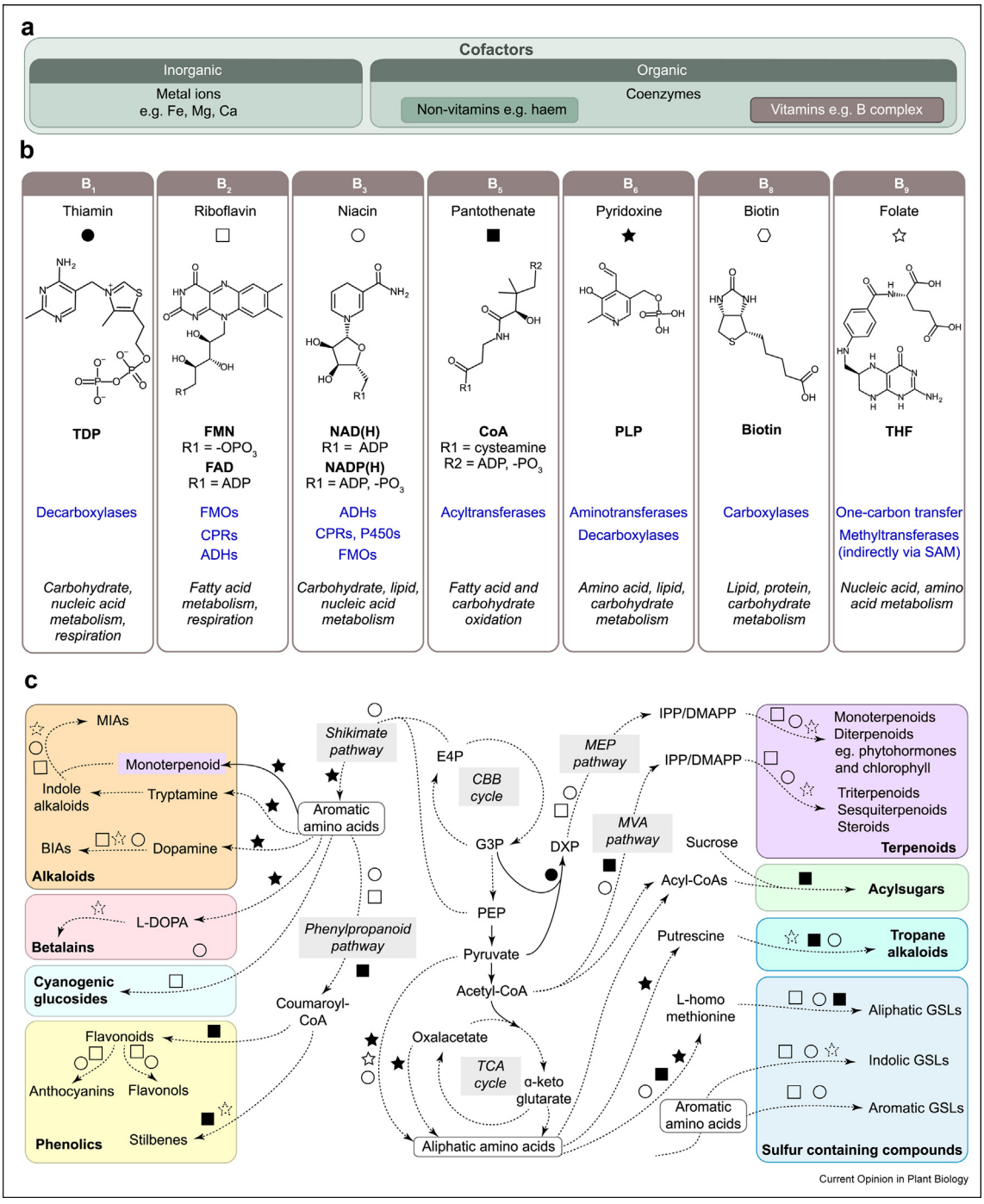
Keywords

Coenzymes, B vitamins, Primary metabolism, Specialized metabolism, Enzymes.

Introduction

Plant natural products (specialized metabolites) are a rich source for drugs, including anticancer, antibiotic, and antifungal therapies—as well as biofuels, cosmetics, perfumes, and flavors. Biological pathways to the biosynthesis of specialized metabolites in plants are considered to have evolved due to selective pressure favored by particular environmental adaptive strategies and thus have been categorized as important for plant–environment interactions [1]. This distinguishes them from primary metabolites which are categorized as essential for fundamental plant growth and development. With unravelling of the biological pathways to the biosynthesis of specialized metabolites, it is clear that many of the enzymes involved utilize coenzymes (accessory organic cofactors required for catalysis, e.g. the vitamin B complex) (Figure 1a–c). However, coenzymes are usually only considered essential to primary metabolism since they are required for plant growth and development [2–9]. The interface of primary and specialized metabolism has received increasing attention over recent years, in particular in the context of evolution of specialized metabolic enzymes from primary metabolic enzymes and the co-regulation of primary metabolic pathways with specialized metabolic pathways. It has recently been pointed out by Erb and Kliebenstein that the classically applied precise biochemical boundaries between a primary metabolite (directly required for plant growth) and a specialized metabolite (involved in plant–environment interactions) are blurred [10]. This is because specialized metabolites also appear to impact plant growth (as for primary metabolites) and can be recycled as precursors of primary metabolites [10–13]. Thus, it has been proposed to view primary and specialized metabolites as integrated whole metabolic networks shaped by environmental selection [10]. This broader definition can also be applied to coenzymes that cannot be simply regarded as part of primary or specialized metabolism alone (Figure 1c). Coenzyme supply must be sufficient for both primary and specialized metabolism and must be assured in circumstances where specialized metabolic pathways are up-regulated, e.g. upon herbivore or pathogen attack or in specialized cell types. However, how coenzyme availability is coordinated in these situations remains largely unknown. Knowledge on the use and regulation of coenzymes by plants has the potential to inform how plants capitalize their ability to capture energy from the sun to produce primary growth products as well as develop taxonomically restricted specialized metabolites that permit fitness and survival in environmental niches. In this review, we outline what can be deciphered from the recent literature with regards to coenzyme use and regulation for specialized metabolism and its integration with the requirements of primary

Figure 1



B vitamins and their importance for specialized metabolism. **(a)** B vitamins are one class of organic cofactors. **(b)** B vitamins are chemically diverse compounds from which active coenzyme forms are derived (structures and names in bold) that are involved in a variety of enzyme reactions (blue) in metabolism (italics). **(c)** A selection of primary metabolic pathways providing precursors for diverse specialized metabolites (shown within colored boxes). Symbols from coenzymes in **(b)** indicate which coenzymes are involved in which pathways. Dashed lines symbolize multi-step reactions. The dashed outlined star depicts indirect dependence on folate via SAM. Abbreviations: Alcohol dehydrogenase (ADH), adenosine diphosphate (ADP), benzoisoquinoline alkaloids (BIAs), Calvin-Benson-Bassham (CBB), coenzyme A (CoA), cytochrome P450 (P450), cytochrome P450 reductases (CPR), dimethylallyl pyrophosphate (DMAPP), erythrose 4′-phosphate (E4P), Flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), flavin dependent monooxygenase (FMO), glyceraldehyde 3′-phosphate (G3P), isopentenyl pyrophosphate (IPP), L-3,4-dihydroxyphenylalanine (L-DOPA), 2-C-methyl-D-erythritol 4-phosphate (MEP), mevalonic acid (MVA), monoterpenoid indole alkaloid (MIA), phosphoenolpyruvate (PEP), pyridoxal 5′-phosphate (PLP), *S*-adenosylmethionine (SAM), tetrahydrofolate (THF), thiamine diphosphate (TDP), trichloroacetic acid (TCA).

metabolism. We emphasize that supply of coenzymes cannot continue to be ignored as it can have an enormous influence on demand and thus the outcome of engineered metabolic pathways, which is particularly important in the era of synthetic biology.

Directly or indirectly, specialized metabolic pathways depend on multiple coenzymes

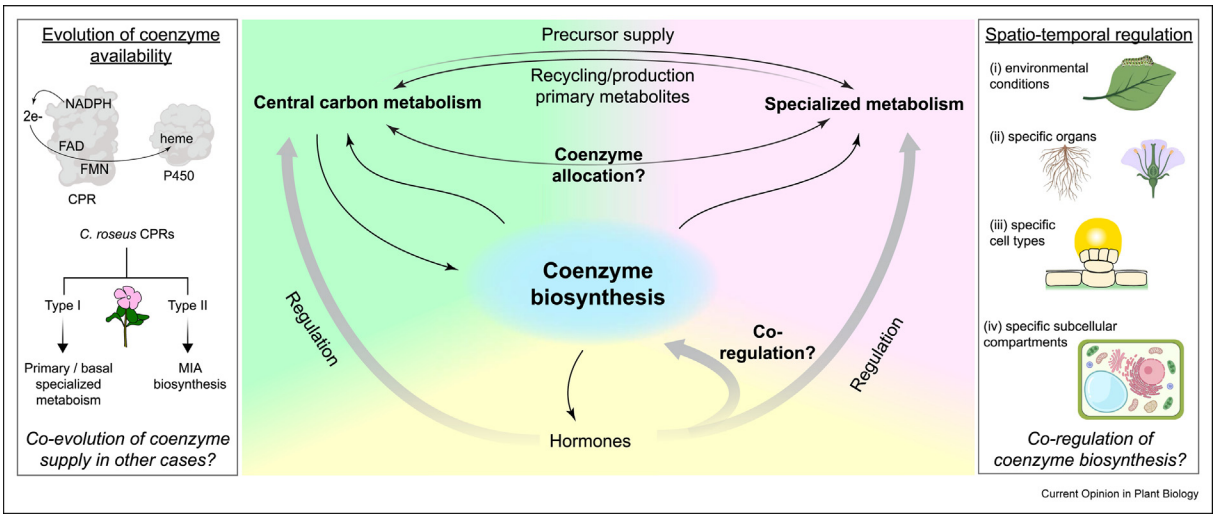
In this review we will focus on the B vitamin complex (B_1—B_9 in plants) as they encompass a family of coenzymes that are involved in a substantial proportion of metabolic reactions (Figure 1b) (see Ref. [14] for an extensive overview of these processes). Notably, many enzymes involved in metabolism utilize other organic cofactors such as lipoic acid and inorganic cofactors such as metal ions or phosphate but will not be discussed here. On the one hand, the B vitamin family are major actors in primary metabolism being involved in protein, carbohydrate, lipid and nucleic acid processes, while on the other hand they facilitate reactions for the main classes (terpenoids, alkaloids, phenolics) of specialized metabolites (Figure 1c). Thus, the role of the B vitamin family cannot be strictly categorized as primary or specialized metabolism specific. The role of B vitamin derived coenzymes in specialized metabolism can be direct or indirect. Direct implies that the coenzyme is necessary for the enzyme catalyzing the formation of the specialized metabolite in question, whereas indirect implies that the coenzyme is necessary for the production of a precursor or of another coenzyme that an enzyme depends on. Select examples serve to illustrate the essential role of B vitamin coenzymes in metabolic networks from which primary and specialized metabolites are derived (Figure 1c).

Redox reactions are likely the most frequently occurring reactions in specialized metabolism and are carried out by oxidoreductases such as alcohol dehydrogenases (ADHs), flavin dependent monooxygenases (FMOs) or cytochrome P450 enzymes (P450s). Enzymes of these classes are involved in the biosynthesis of a dazzling array of specialized metabolites spanning phenylpropanoids, terpenoids, cyanogenic glycosides and alkaloids, in addition to primary metabolism categorized phytohormone biosynthesis. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) (derived from vitamin B_3, niacin) is required for the transfer of electrons that occurs within the catalytic mechanism of P450s. The source of electrons is usually coupled to P450 reductases (CPRs) which in turn rely on the reduced coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) (derived from vitamin B_2, riboflavin) to transfer two electrons from NADPH (Figure 1b) to the haem coenzyme of P450s [15] (Figure 2, left panel). FAD and FMN are also common coenzymes for other classes of redox enzymes such as ADHs and berberine bridge enzymes involved in alkaloid biosynthesis [16,17]. Coenzyme A (CoA) (derived from vitamin B_5, pantothenate) functions as an acyl group carrier a common moiety on specialized metabolites, e.g. acylsugars [18,19] (Figure 1b and c). The versatile coenzyme PLP (derived from vitamin B_6) can directly act in diverse enzymatic reactions spanning both primary (e.g. amino acid biosynthesis) and specialized metabolism (e.g. allicin biosynthesis). As amino acids are precursors of specialized metabolism, this is an example of indirect dependency of specialized metabolism on B vitamins [20] (Figure 1b and c). PLP is also necessary for enzymes at the interface of primary and specialized metabolism, e.g. aromatic amino acid decarboxylases such as tryptophan decarboxylase that produces the indole tryptamine for alkaloid biosynthesis and tyrosine/L-DOPA decarboxylase yielding tyramine or dopamine, precursors for betalain and benzylisoquinoline alkaloid (BIA) biosynthesis among others [21] (Figure 1b and c). B vitamin derived coenzymes spanning both primary and specialized metabolism also include methyltransferase reactions, where the methyl is derived from *S*-adenosylmethionine (SAM) whose biosynthesis is in turn dependent on folate (vitamin B_9) [22] (Figure 1b and c). Vitamin B_1, in its form as thiamine diphosphate (TDP) is an essential coenzyme for several primary key energy generating reactions in both photosynthesis (e.g. transketolase) and respiration (e.g. α-ketoglutarate dehydrogenase), as well as deoxyxylulose 5-phosphate (DXP) synthesis (Figure 1b and c). DXP is precursor in one (2-C-methyl-D-erythritol 4-phosphate (MEP)) of two pathways (the other uses mevalonate (MVA)) to the biosynthesis of isoprenoids, the most ancient class of specialized products [23]. The isoprenoids in turn comprise essential plant pigments such as chlorophyll as well as certain phytohormones, in addition to an assortment of mono- (10C), sesqui-(15C) and di- (20C) and tri-(30C) terpenoid plant defense compounds (Figure 1c). Notably, TDP is also essential for folate biosynthesis and thus indirectly essential for methyltransferases as well. Thus, there is a high level of interdependence and ultimately specialized metabolic pathways depend directly or indirectly on coenzymes of the B vitamin complex. There are also instances where coenzymes (or their precursors) can serve as substrates for specialized metabolic pathways, e.g. an isoform of the bifunctional gene (*RibA/B*) involved in riboflavin biosynthesis has recently been implicated in the biosynthesis of the anti-nutritional specialized metabolite, vicine, in faba bean [24]. It would be interesting to establish if harnessing coenzyme biosynthesis genes for specialized metabolite biosynthesis can create competition with coenzyme biosynthesis.

Coordination of coenzyme supply to primary and specialized metabolism deserves attention

Coenzyme supply contributes to the pool of active (holo)enzyme and thereby can affect metabolic flux.

Figure 2



Coordination of coenzyme supply with primary and specialized metabolism. Scheme depicting areas warranting further research. Black arrows indicate coenzyme supply flux, gray arrows indicate regulatory effects such as transcriptional regulation. Box on the left: Mechanism of electron transfer from the NADPH coenzyme of cytochrome P450 reductases (CPRs) to the haem of cytochrome P450s (P450s) via the two flavin coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). In *Catharanthus roseus* (*C. roseus*) there are two classes of CPRs, with class II being specifically associated with monoterpenoid indole alkaloid (MIA) biosynthesis [15]. This is likely an evolutionary adaptation to meet the high demand for coenzymes required by P450 redox reactions in the MIA pathway. It is unknown if similar evolutionary adaptations have occurred to meet coenzyme demand in other pathways and species. Box on the right: Specialized metabolism is highly transcriptionally regulated in a spatio-temporal fashion. To which extent coenzyme biosynthesis is coregulated with specialized metabolism is largely unknown. Some elements of this figure were made with Biorender.

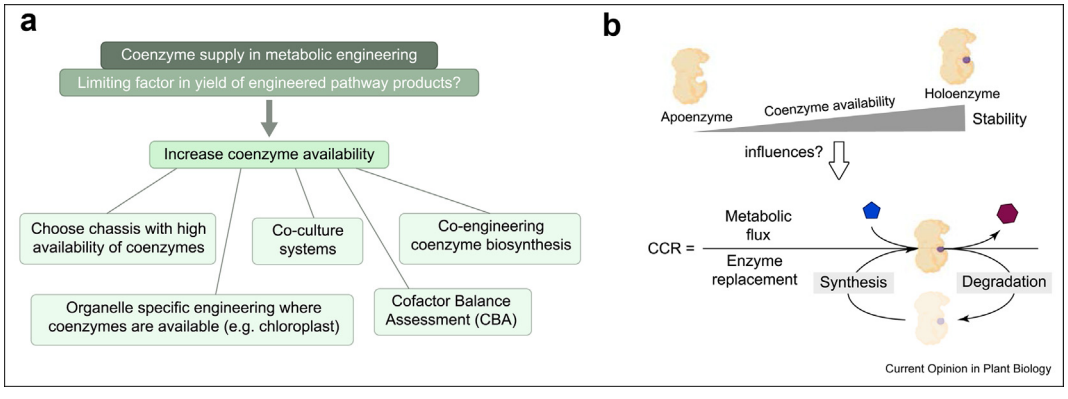
Thus, in the presence of precursors, coenzyme levels have the potential to drive metabolic flux in a particular direction. However, direct evidence for this with regard to specialized metabolic pathway flux is currently lacking. Firstly, coenzymes themselves are derived from compounds of central carbon metabolism (e.g. contributions from amino acids: glycine (B_1), aspartate (B_3), glutamine (B_6) alanine (B_8), among others) or salvaged from non-coenzyme forms and in relatively small amounts. A comprehensive overview of vitamin biosynthesis is given in Ref. [14]. Given the essentiality of coenzymes for enzyme function, one important consideration is how supply is coordinated with the demands of both primary and specialized metabolism. For this, understanding the evolution of specialized metabolism with primary metabolism and the factors regulating carbon flow between primary and secondary metabolism will be required (Figure 2, central panel). One aspect is that coenzyme supply must co-evolve with species-specific specialized metabolic pathways so that coenzyme availability is not a limiting factor. Such evolutionary adaptations have already been shown for precursor supply, for instance non-feedback sensitive prephenate dehydrogenases that coevolved with tyrosine-dependent specialized metabolic pathways in some species [25–27]. There is some evidence that coenzyme biosynthesis, or rather regeneration, co-evolved with specialized metabolism, e.g. in *Catharanthus roseus* there are two types of CPRs (I and II) transferring electrons from NADPH that rely on FMN/FAD as mentioned above, with a predominant role for type II in specialized metabolism (e.g. monoterpenoid indole alkaloid biosynthesis (MIA)) (Figure 2, left panel) [15]. Potential evolutionary adaptations of coenzyme biosynthesis or recycling has not yet been investigated. Other aspects to be considered include ensuring enough coenzyme is available at the spatio-temporal scale, that is when and where specialized metabolism is upregulated such as (i) under certain environmental conditions, e.g. stress; (ii) in specific organs, e.g. flowers; (iii) in specific cell types e.g. glandular trichomes or idioblasts; (iv) in specific subcellular compartments where specialized metabolic enzymes are located or micro-compartments such as metabolons (Figure 2, right panel) [28,29]. Little is known about how these aspects are regulated and how homeostasis is reached such that primary metabolism is not compromised. This could be achieved by upregulation of biosynthesis or transport of the coenzyme (or a precursor) to the corresponding organ, cell type or compartment. For instance, the MEP and MVA pathways generating the isoprenoid building unit isopentenyl phosphate are up-regulated in specialized structures or cell types where the respective specialized metabolic pathways they feed into occur. Examples include the glandular trichomes in tomato or peppermint, the laticifers in the rubber tree, and Internal Phloem Associated Parenchyma (IPAP) cells in *C. roseus* [30–36].

However, coregulation with coenzyme biosynthesis has only been shown for NADPH and in glandular trichomes [30,33]. Coenzyme availability in specialized cell types could also be assured by increased import from other "source" cells. For example, the biosynthesis of acyl-CoA in tomato trichomes implies the availability of CoA in sufficient amounts and it would be interesting to determine if CoA biosynthesis or import is also adjusted [18]. To ensure coenzyme availability at the subcellular level, transport might also be increased but has not yet been shown. It is tempting to speculate that when growth and development are arrested, e.g. stress, coenzymes may become available or are remobilized and allocated to specialized metabolism and thus differential regulation from primary metabolism may not be necessary for sufficient coenzyme supply. Notwithstanding any acquired up-regulation of coenzyme biosynthesis should be tightly controlled since over-accumulation of free coenzymes may have adverse effects or inhibit other regulatory processes as shown for PLP that inhibits the chloroplast envelope triose phosphate transporter [37]. Furthermore, the biosynthesis of some coenzymes is influenced by environmental parameters including light and it is not known if distal biosynthesis affects locally derived products (e.g. shoot derived products influence those of the root). Independent of cellular coenzyme concentration, a first example of a post-translational mechanism to alter coenzyme supply was recently provided. The phosphorylation status of an active site threonine residue of tryptophan aminotransferase of Arabidopsis 1 (TAA1), a key enzyme in auxin biosynthesis, was shown to act as a switch, which in the "on" state prevents PLP binding and thus enzyme activity [38]. Thus, coordination of coenzyme supply and specialized metabolism in a spatio-temporal manner deserves more investigation.

Coenzyme supply in metabolic engineering

The knowledge base on specialized metabolites has triggered the burgeoning field of metabolic engineering/synthetic biology, where cells (as biocatalysts) house the designed synthesis of compounds of medicinal, industrial or scientific interest. Synthetic biology has the potential to replace chemicals sourced from unsustainable natural sources and/or inspire the design of new-to-nature compounds that cannot be produced by classical chemistry techniques. During the design process it is important to take into account the natural ability of the cell and the impact of the production of an artificially designed product on homeostasis. Rarely has coenzyme supply been considered in the iterative design process or tested in metabolic engineering approaches. This disregard is possibly because coenzyme regulation is not understood and moreover, has not yet received the attention it deserves. Nonetheless, for optimization of engineered biocatalysts, constraints imposed by coenzyme availability need to be identified and considered (Figure 3a) [39,40]. In *Escherichia coli*, addition of PLP to the culture expressing the PLP-dependent phenylacetaldehyde synthase increased to some extent the production of 2-phenylethanol, used as rose scent in the food and fragrance industry [41]. Further, engineering increased NADPH availability enhanced strictosidine and noscapine biosynthesis in yeast [42,43]. While this limited number of examples suggests that coenzyme availability is a constraint in pathway engineering more research is needed to determine how common this is. Constraints may be divergent across host species used as biosynthesis platforms (i.e. chassis) and are dependent on the coenzyme requirement of the enzymes used (Figure 3a). Co-culture systems could be used for the supply of coenzymes to chassis systems as long as limitations in transport are considered and growth of

Figure 3



Coenzyme availability in metabolic engineering and concept of Catalytic Cycles until Replacement. **(a)** Aspects to consider with regard to coenzyme supply in metabolic engineering approaches. **(b)** Enzymes can perform a specific number of catalytic cycles until they are replaced [47,48]. Coenzymes make enzymes generally more stable and their availability may therefore hypothetically influence the "Catalytic Cycles until Replacement" (CCR) of a given enzyme. Some elements of this figure were made with Biorender.

multiple species is facilitated (Figure 3a). For example, benzylisoquinoline alkaloids were synthesized in an *E. coli* and yeast co-culture system [44] that facilitated sharing of the metabolic burden while utilizing the species most suited to expression of the required enzymes. However, the ultimate chassis may be a plant cell and the use of a transient plant model system such as tobacco (*Nicotiana benthamiana*) is well established due to conservation of specific cell compartmentation and protein processing. Moreover, coenzyme use is likely to be preserved even in a heterologous plant system. Coenzyme supply may become a limiting factor when making specialized metabolites constitutively in a host plant and remains to be evaluated. Although, engineering artemisinic acid production in tobacco chloroplasts appeared to work efficiently and may be because redox coenzymes are available in high quantities in this organelle and are needed for the enzymes of this pathway (e.g. P450s) [45]. In addition, the recent introduction of a "Cofactor Balance Assessment (CBA)" for ATP and NAD(P)H usage and its impact on yield efficiency of butanol production in *E. coli* [46] could be extended to the use of coenzymes in other (bio)synthetic pathways and in plants. In this way the accommodation and performance of pathways in terms of coenzyme usage could be assessed.

Enzyme turnover should be considered

While there is a wealth of literature on metabolic engineering of biomolecules, the lifetime of the catalytic units, enzymes, has classically not been taken into account for their overall metabolic output. This is important because parameters such as functional duration or factors driving replacement of enzymes can greatly impact performance due to the drain on energy but are not known in most cases to date [47]. Thus, it has recently been proposed to incorporate a metric *Catalytic Cycles until Replacement* (CCR) to aid in productivity estimates in terms of protein components [48]. CCR is expressed as the metabolic flux rate over the protein turnover rate (Figure 3b). Estimates of CCRs range from $<10^3$ to $>10^7$ with enzymes that involve substrates, intermediates or products that can attack reactive amino acid residues having the lowest values [47]. Taking CCR into account combined with strategies to raise values and lower replacement costs of the enzymes being used is proposed to greatly improve performance and efficiency of engineering systems for synthetic biology. This concept could be extended to include supply, repair and turnover of the corresponding coenzymes (where used) as fuel driving duration of enzyme activity and repurposing upon degradation of the corresponding enzyme. Holoenzymes are typically more stable than the respective apoenzyme [49], thus coenzyme biosynthesis and assembly can greatly influence CCR (Figure 3b). For example, enzymes dependent on TDP for catalysis may be constrained by the supply of this coenzyme [50]. Further, two of the proteins involved in its biosynthesis, THIC and THI1, are among those with the shortest protein half-lives reported and must be constantly (re) synthesized [2,51,52]. Moreover, a backbone S atom of THI1 is used within the biosynthesis of TDP rendering the protein catalytically useless after a single cycle and imposing a further energy draining limitation for its constant replacement. Thus, engineering of a more catalytically efficient THI1 capable of multiple turnovers could decrease the drain of cellular energy, release the limitation of coenzyme supply and possibly increase the value of CCR of the corresponding dependent enzymes. Furthermore, the possibility of engineering more stable synthetic coenzymes and/or the more efficient pathways to supply them could be considered.

Expanding knowledge bases to include coenzyme information

As genomic resources continue to expand rapidly, coupled with increasing knowledge on the relationship between structure and function, our ability to mine databases for enzymes that use coenzymes is enabled. The UniProt knowledge base (UniProtKB) which is a comprehensive, high-quality, and freely accessible resource of protein sequences and functional annotation that covers genomes and proteomes from tens of thousands of taxa, including a broad range of plants and microorganisms producing natural products of medical, nutritional, and agronomical interest has recently incorporated a feature that links chemical structures of products to enzyme reactions [53]. Another excellent resource for plant metabolic pathways is the Plantcyc (PMN) database [54] that shows detailed reaction schemes including molecular structures. In the chemical reaction schemes of these databases the oxidation or reduction of redox enzymes such as NAD(P)H are depicted, but dependence on other coenzymes is not shown. For instance, tryptophan decarboxylase is a PLP-dependent enzyme which is not indicated in the reaction schemes and can only be found in the binding site prediction on UniProt but is not currently visible on PMN. The inclusion of coenzymes would increase the awareness of their importance for metabolic pathways. Additionally, it would be helpful to be able to immediately have a list of coenzymes necessary for a metabolic pathway of interest. Moreover, this can be coupled with structural features that manifest distinct substrate and mechanistic selectivity to facilitate the biosynthesis of a specialized product as has recently been shown in the case of PLP-dependent L-amino acid decarboxylases [21].

Conclusion

Future work should aim at defining coenzyme supply with demand and its coordination between metabolic networks as a function of the environment. This will not only improve our knowledge on the importance of these factors but also assist in developing efficient metabolic

engineering strategies. Information on coenzyme metabolism can be harnessed to provide a versatile toolset for such strategies to allow plant survival in a particular perhaps "uncharted" environment in addition to being a possible source of human medicines and commodities. Moreover, an understanding of coenzyme metabolism would be very useful and informative to computationally predict the outcome of engineered biosynthetic pathways and the maintenance of metabolic homeostasis and growth, culling the need for extensive experimental determination during the design/build/test iterations used in engineering approaches.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Financial support is gratefully acknowledged from the Swiss National Science Foundation (Grant 31003A-141117/1 and 310030-192466 to T.B.F.) as well as the University of Geneva. We thank the anonymous reviewers for their valuable comments leading to improvement of the manuscript.

References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Weng J-K, Lynch JH, Matos JO, Dudareva N: **Adaptive mechanisms of plant specialized metabolism connecting chemistry to function**. *Nat Chem Biol* 2021, **17**:1037–1045.

2. Raschke M, Burkle L, Muller N, Nunes-Nesi A, Fernie AR, Arigoni D, Amrhein N, Fitzpatrick TB: **Vitamin B1 biosynthesis in plants requires the essential iron sulfur cluster protein, THIC**. *Proc Natl Acad Sci USA* 2007, **104**:19637–19642.

3. Tambasco-Studart M, Titiz O, Raschle T, Forster G, Amrhein N, Fitzpatrick TB: **Vitamin B6 biosynthesis in higher plants**. *Proc Natl Acad Sci USA* 2005, **102**:13687–13692.

4. Titiz O, Tambasco-Studart M, Warzych E, Apel K, Amrhein N, Laloi C, Fitzpatrick TB: **PDX1 is essential for vitamin B6 biosynthesis, development and stress tolerance in Arabidopsis**. *Plant J* 2006, **48**:933–946.

5. Katoh A, Uenohara K, Akita M, Hashimoto T: **Early steps in the biosynthesis of NAD in Arabidopsis start with aspartate and occur in the plastid**. *Plant Physiol* 2006, **141**:851–857.

6. Sa N, Rawat R, Thornburg C, Walker KD, Roje S: **Identification and characterization of the missing phosphatase on the riboflavin biosynthesis pathway in *Arabidopsis thaliana***. *Plant J* 2016, **88**:705–716.

7. Rubio S, Larson TR, Gonzalez-Guzman M, Alejandro S, Graham IA, Serrano R, Rodriguez PL: **An Arabidopsis mutant impaired in coenzyme A biosynthesis is sugar dependent for seedling establishment**. *Plant Physiol* 2006, **140**:830–843.

8. Ishikawa T, Machida C, Yoshioka Y, Kitano H, Machida Y: **The *GLOBULAR ARREST1* gene, which is involved in the biosynthesis of folates, is essential for embryogenesis in *Arabidopsis thaliana***. *Plant J* 2003, **33**:235–244.

9. Schneider T, Dinkins R, Robinson K, Shellhammer J, Meinke DW: **An embryo-lethal mutant of *Arabidopsis thaliana* is a biotin auxotroph**. *Dev Biol* 1989, **131**:161–167.

10. •• Erb M, Kliebenstein DJ: **Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy**. *Plant Physiol* 2020, **184**:39–52.
This excellent review emphasizes that the classical boundary between specialized metabolites and the other metabolite classes, primary metabolites and hormones, is in fact blurry. Specialized metabolites are multi-functional and can act as growth regulators or feed into primary metabolism in addition to mediating plant–environment interactions.

11. Malinovsky FG, Thomsen MF, Nintemann SJ, Jagd LM, Bourgine B, Burow M, Kliebenstein DJ: **An evolutionarily young defense metabolite influences the root growth of plants via the ancient TOR signaling pathway**. *Elife* 2017, **6**.

12. Soubeyrand E, Johnson TS, Latimer S, Block A, Kim J, Colquhoun TA, Butelli E, Martin C, Wilson MA, Basset GJ: **The peroxidative cleavage of kaempferol contributes to the biosynthesis of the benzenoid moiety of ubiquinone in plants**. *Plant Cell* 2018, **30**:2910–2921.

13. Sugiyama R, Li R, Kuwahara A, Nakabayashi R, Sotta N, Mori T, Ito T, Ohkama-Ohtsu N, Fujiwara T, Saito K, *et al.*: **Retrograde sulfur flow from glucosinolates to cysteine in *Arabidopsis thaliana***. *Proc Natl Acad Sci USA* 2021, **118**, e2017890118.

14. Rebeille F, Douce R: *Biosynthesis of vitamins in plants*. Ireland: Academic Press; 2011.

15. • Parage C, Foureau E, Kellner F, Burlat V, Mahroug S, Lanoue A, Duge de Bernonville T, Londono MA, Carqueijeiro I, Oudin A, *et al.*: **Class II cytochrome P450 reductase governs the biosynthesis of alkaloids**. *Plant Physiol* 2016, **172**:1563–1577.
The authors show that in *C. roseus* there is an additional class of CPRs that specifically reduce P450s involved in MIA biosynthesis (via the coenzymes NADPH, FAD, FMN). This appears to be an evolutionary adaptation of coenzyme availability via CPRs to meet the high demand of MIA biosynthesis for reducing power.

16. Daniel B, Konrad B, Toplak M, Lahham M, Messenlehner J, Winkler A, Macheroux P: **The family of berberine bridge enzyme-like enzymes: a treasure-trove of oxidative reactions**. *Arch Biochem Biophys* 2017, **632**:88–103.

17. Eggers R, Jammer A, Jha S, Kerschbaumer B, Lahham M, Strandback E, Toplak M, Wallner S, Winkler A, Macheroux P: **The scope of flavin-dependent reactions and processes in the model plant *Arabidopsis thaliana***. *Phytochemistry* 2021, **189**:112822.

18. Fan P, Wang P, Lou Y-R, Leong BJ, Moore BM, Schenck CA, Combs R, Cao P, Brandizzi F, Shiu S-H, *et al.*: **Evolution of a plant gene cluster in *Solanaceae* and emergence of metabolic diversity**. *Elife* 2020, **9**, e56717.

19. Moghe GD, Leong BJ, Hurney SM, Daniel Jones A, Last RL: **Evolutionary routes to biochemical innovation revealed by integrative analysis of a plant-defense related specialized metabolic pathway**. *Elife* 2017, **6**, e28468.

20. Hoffarth ER, Rothchild KW, Ryan KS: **Emergence of oxygen- and pyridoxal phosphate-dependent reactions**. *FEBS J* 2020, **287**:1403–1428.

21. Torrens-Spence MP, Chiang Y-C, Smith T, Vicent MA, Wang Y, Weng J-K: **Structural basis for divergent and convergent evolution of catalytic machineries in plant aromatic amino acid decarboxylase proteins**. *Proc Natl Acad Sci USA* 2020, **117**:10806–10817.

22. Herbig K, Chiang E-P, Lee L-R, Hills J, Shane B, Stover PJ: **Cytoplasmic serine hydroxymethyltransferase mediates competition between folate-dependent deoxyribonucleotide and *S*-adenosylmethionine biosyntheses**. *J Biol Chem* 2002, **277**:38381–38389.

23. Lange BM: **The evolution of plant secretory structures and emergence of terpenoid chemical diversity**. *Annu Rev Plant Biol* 2015, **66**:139–159.

24. •• Björnsdotter E, Nadzieja M, Chang W, Escobar-Herrera L, Mancinotti D, Angra D, Xia X, Tacke R, Khazaei H, Crocoll C, *et al.*: **VC1 catalyses a key step in the biosynthesis of vicine in faba bean**. *Native Plants* 2021, **7**:923–931.
In this elegant work the vicine biosynthesis pathway is discovered which unexpectedly revealed that a key biosynthesis enzyme is presumably harnessed from the biosynthesis pathway of the coenzyme riboflavin.

25. Schenck CA, Chen S, Siehl DL, Maeda HA: **Non-plastidic, tyrosine-insensitive prephenate dehydrogenases from legumes**. *Nat Chem Biol* 2015, **11**:52–57.

26. Schenck CA, Holland CK, Schneider MR, Men Y, Lee SG, Jez JM, Maeda HA: **Molecular basis of the evolution of alternative tyrosine biosynthetic routes in plants**. *Nat Chem Biol* 2017, **13**:1029–1035.

27. Lopez-Nieves S, Yang Y, Timoneda A, Wang M, Feng T, Smith SA, Brockington SF, Maeda HA: **Relaxation of tyrosine pathway regulation underlies the evolution of betalain pigmentation in Caryophyllales**. *New Phytol* 2018, **217**:896–908.

28. Laursen T, Borch J, Knudsen C, Bavishi K, Torta F, Martens HJ, Silvestro D, Hatzakis NS, Wenk MR, Dafforn TR, *et al.*: **Characterization of a dynamic metabolon producing the defense compound dhurrin in sorghum**. *Science* 2016, **354**:890–893.

29. Colinas M, Goossens A: **Combinatorial transcriptional control of plant specialized metabolism**. *Trends Plant Sci* 2018, **23**:324–336.

30. •• Balcke GU, Bennewitz S, Bergau N, Athmer B, Henning A, Majovsky P, Jiménez-Gómez JM, Hoehenwarter W, Tissier A: **Multi-omics of tomato glandular trichomes reveals distinct features of central carbon metabolism supporting high productivity of specialized metabolites**. *Plant Cell* 2017, **29**:960–983.

While adaptations of primary metabolism to support specialized metabolism have been shown in other work, this very comprehensive study additionally revealed that the biosynthesis of the coenzyme NADPH is also up-regulated, showing co-regulation of specialized metabolism and coenzyme biosynthesis.

31. Schilmiller AL, Miner DP, Larson M, McDowell E, Gang DR, Wilkerson C, Last RL: **Studies of a biochemical factory: tomato trichome deep expressed sequence tag sequencing and proteomics**. *Plant Physiol* 2010, **153**:1212–1223.

32. Conneely LJ, Mauleon R, Mieog J, Barkla BJ, Kretzschmar T: **Characterization of the *Cannabis sativa* glandular trichome proteome**. *PLoS One* 2021, **16**, e0242633.

33. Johnson SR, Lange I, Srividya N, Lange BM: **Bioenergetics of monoterpenoid essential oil biosynthesis in nonphotosynthetic glandular trichomes**. *Plant Physiol* 2017, **175**:681–695.

34. Soetaert SS, Van Neste CM, Vandewoestyne ML, Head SR, Goossens A, Van Nieuwerburgh FC, Deforce DL: **Differential transcriptome analysis of glandular and filamentous trichomes in *Artemisia annua***. *BMC Plant Biol* 2013, **13**:220.

35. Tan D, Hu X, Fu L, Kumpeangkeaw A, Ding Z, Sun X, Zhang J: **Comparative morphology and transcriptome analysis reveals distinct functions of the primary and secondary laticifer cells in the rubber tree**. *Sci Rep* 2017, **7**:3126.

36. Guirimand G, Guihur A, Perello C, Phillips M, Mahroug S, Oudin A, Dugé de Bernonville T, Besseau S, Lanoue A, Giglioli-Guivarc'h N, *et al.*: **Cellular and subcellular compartmentation of the 2C-methyl-D-erythritol 4-phosphate pathway in the Madagascar periwinkle**. *Plants* 2020, **9**:462.

37. Gross A, Brückner G, Heldt HW, Flügge U-I: **Comparison of the kinetic properties, inhibition and labelling of the phosphate translocators from maize and spinach mesophyll chloroplasts**. *Planta* 1990:180.

38. Wang Q, Qin G, Cao M, Chen R, He Y, Yang L, Zeng Z, Yu Y, Gu Y, Xing W, *et al.*: **A phosphorylation-based switch controls TAA1-mediated auxin biosynthesis in plants**. *Nat Commun* 2019, **11**:679.

39. Cravens A, Payne J, Smolke CD: **Synthetic biology strategies for microbial biosynthesis of plant natural products**. *Nat Commun* 2019, **10**:2142.

40. • Chen R, Yang S, Zhang L, Zhou YJ: **Advanced strategies for production of natural products in yeast**. *iScience* 2020, **23**:100879.

This review provides an excellent overview about pathway engineering in yeast with a particular focus on cofactor engineering strategies.

41. Achmon Y, Ben-Barak Zelas Z, Fishman A: **Cloning Rosa hybrid phenylacetaldehyde synthase for the production of 2-phenylethanol in a whole cell *Escherichia coli* system**. *Appl Microbiol Biotechnol* 2014, **98**:3603–3611.

42. Brown S, Clastre M, Courdavault V, O'Connor SE: ***De novo* production of the plant-derived alkaloid strictosidine in yeast**. *Proc Natl Acad Sci USA* 2015, **112**:3205–3210.

43. Li Y, Li S, Thodey K, Trenchard I, Cravens A, Smolke CD: **Complete biosynthesis of noscapine and halogenated alkaloids in yeast**. *Proc Natl Acad Sci USA* 2018, **115**:E3922–E3931.

44. Minami H, Kim J-S, Ikezawa N, Takemura T, Katayama T, Kumagai H, Sato F: **Microbial production of plant benzylisoquinoline alkaloids**. *Proc Natl Acad Sci USA* 2008, **105**:7393.

45. Fuentes P, Zhou F, Erban A, Karcher D, Kopka J, Bock R: **A new synthetic biology approach allows transfer of an entire metabolic pathway from a medicinal plant to a biomass crop**. *Elife* 2016, **5**, e13664.

46. • de Arroyo Garcia L, Jones PR: ***In silico* co-factor balance estimation using constraint-based modelling informs metabolic engineering in *Escherichia coli***. *PLoS Comput Biol* 2020, **16**, e1008125.

The authors developed a computational approach to quantify the cofactor balance of a metabolic engineering design. Optimizing cofactor balance may increase target production in metabolic engineering.

47. •• Hanson AD, McCarty DR, Henry CS, Xian X, Joshi J, Patterson JA, García-García JD, Fleischmann SD, Tivendale ND, Millar AH: **The number of catalytic cycles in an enzyme's lifetime and why it matters to metabolic engineering**. *Proc Natl Acad Sci USA* 2021, **118**, e2023348118.

This work provides for the first-time estimations of catalytic cycles until replacement (CCRs) for hundreds of proteins from three species from different kingdoms including Arabidopsis. Increasing the CCR number through enzyme engineering will likely make specialized metabolic pathway engineering more productive.

48. Tivendale ND, Hanson AD, Henry CS, Hegeman AD, Millar AH: **Enzymes as parts in need of replacement – and how to extend their working life**. *Trends Plant Sci* 2020, **25**:661–669.

49. Martínez-Limón A, Alriquet M, Lang W-H, Calloni G, Wittig I, Vabulas RM: **Recognition of enzymes lacking bound cofactor by protein quality control**. *Proc Natl Acad Sci USA* 2016, **113**:12156–12161.

50. Fitzpatrick TB, Chapman LM: **The importance of thiamine (vitamin B1) in plant health: from crop yield to biofortification**. *J Biol Chem* 2020, **295**:12002–12013.

51. Chatterjee A, Abeydeera ND, Bale S, Pai P-J, Dorrestein PC, Russell DH, Ealick SE, Begley TP: ***Saccharomyces cerevisiae* THI4p is a suicide thiamine thiazole synthase**. *Nature* 2011, **478**:542–546.

52. Joshi J, Beaudoin GAW, Patterson JA, García-García JD, Belisle CE, Chang L-Y, Li L, Duncan O, Millar AH, Hanson AD: **Bioinformatic and experimental evidence for suicidal and catalytic plant THI4s**. *Biochem J* 2020, **477**:2055–2069.

53. Feuermann M, Boutet E, Morgat A, Axelsen K, Bansal P, Bolleman J, de Castro E, Coudert E, Gasteiger E, Géhant S, *et al.*: **Diverse taxonomies for diverse chemistries: enhanced representation of natural product metabolism in UniProtKB**. *Metabolites* 2021, **11**:48.

54. Schlapfer P, Zhang P, Wang C, Kim T, Banf M, Chae L, Dreher K, Chavali AK, Nilo-Poyanco R, Bernard T, *et al.*: **Genome-wide prediction of metabolic enzymes, pathways, and gene clusters in plants**. *Plant Physiol* 2017, **173**:2041–2059.