Engineering Plant Secondary Metabolism in Microbial Systems[1][OPEN]

Michael E. Pyne, Lauren Narcross, and Vincent J.J. Martin[2,3]

Department of Biology, Centre for Applied Synthetic Biology, Centre for Structural and Functional Genomics, Concordia University, Montreal, Quebec, Canada

ORCID ID: 0000-0001-7511-115X (V.J.J.M.).

Secondary metabolites are broadly defined as natural products synthesized by an organism that are not essential to support growth and life. The plant kingdom manufactures over 200,000 distinct chemical compounds, most of which arise from specialized metabolism. While these compounds play important roles in interspecies competition and defense, many plant natural products have been exploited for use as medicines, fragrances, flavors, nutrients, repellants, and colorants.

Despite this vast chemical diversity, many secondary metabolites are present at very low concentrations in planta, eliminating crop-based manufacturing as a means of attaining these important products. The structural and stereochemical complexity of specialized metabolites hinders most attempts to access these compounds using chemical synthesis. Although native plants can be engineered to accumulate target pathway metabolites (Zhou et al., 2009; Glenn et al., 2013; Lange and Ahkami, 2013; Wilson and Roberts, 2014; Tatsis and O'Connor, 2016), metabolic engineering is technically more challenging in plants than in microbes.

Advancements in synthetic biology have stimulated the synthesis of valuable natural products in tractable laboratory microbes by interfacing plant secondary pathways with core host metabolism. Microbial synthesis overcomes many of the obstacles hindering traditional chemical synthesis and plant metabolic engineering, thus providing an alternative avenue for exploring plant specialized pathways.

This Update provides a brief overview of engineering plant secondary metabolism in microbial systems. We briefly outline biosynthetic pathways mediating formation of the major classes of natural products with an emphasis on high-value terpenoids, alkaloids, phenylpropanoids, and polyketides. We also highlight common themes, strategies, and challenges underlying efforts to reconstruct and engineer these pathways in microbial hosts. We focus chiefly on de novo biosynthetic approaches in which plant specialized metabolites are synthesized directly from sugar feedstocks rather than supplemented precursors or intermediates. Readers are directed to a selection of pioneering supplementation studies within the context of microbially sourced plant natural products (Becker et al., 2003; Kaneko et al., 2003; Yan et al., 2005; Watts et al., 2006; Leonard et al., 2007, 2008; Hawkins and Smolke, 2008; Fossati et al., 2014, 2015).

OVERVIEW OF KEY PLANT SECONDARY METABOLIC PATHWAYS

Terpenoids

Terpenoids (also called isoprenoids) are the largest class of plant secondary metabolites, comprising more than 50,000 natural products (Connolly and Hill, 1991).

ADVANCES

- Microbial synthesis of plant secondary metabolites is in the midst of a renaissance.
- The elucidation of major plant biosynthetic routes has stimulated the production of high-value natural products in microbial systems.
- Efforts are expanding beyond precursors and branch point intermediates to functionalized end products.
- Although baker's yeast (*Saccharomyces cerevisiae*) remains the host of choice for reconstructing plant secondary pathways, the range of alternative hosts has diversified.
- Synthetic biology has enabled the expansion of the plant secondary metabolic space in microbial species.

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[2]Author for contact: vincent.martin@concordia.ca.

[3]Senior author.

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Whereas the terpene classification refers strictly to hydrocarbons, terpenoids possess a range of chemical functionalities. The central precursors geranyl pyrophosphate (GPP; C_{10}), farnesyl pyrophosphate (FPP; C_{15}), and geranylgeranylpyrophosphate (GGPP; C_{20}) form the structural basis of most higher order terpenoids (Fig. 1). Plant terpene synthases convert these pyrophosphate intermediates to terpenes, which are then functionalized in downstream reactions.

GPP forms the backbone of most monoterpenoids (C_{10}), including linear (geraniol and linalool) and cyclic (camphor and eucalyptol) terpenoids, as well as monoterpenes (limonene and pinene). Geraniol can be modified to the pest repellant citronellol, while limonene gives rise to menthol, a flavoring agent and decongestant. GPP also supplies the prenyl group in the biosynthesis of cannabinoids (Ahmed et al., 2015; Vickery et al., 2016), a class of natural products with promising pharmaceutical properties (Aizpurua-Olaizola et al., 2016). Sesquiterpenes (C_{15}) are derived from FPP, itself generated through condensation of GPP and isopentenyl pyrophosphate (IPP) in yeast (*Saccharomyces cerevisiae*) or directly from IPP and dimethylallyl pyrophosphate units in plants. The sesquiterpene amorphadiene gives rise to the antimalarial drug precursor artemisinic acid. The addition of another IPP unit to FPP in yeast gives rise to GGPP, which forms the scaffold of the diterpenes and diterpenoids (C_{20}), such as taxadiene, a precursor to the taxane family of chemotherapeutics. Condensation of two FPP units yields the linear triterpene squalene (C_{30}), which serves as the universal building block of all sterols, including ergosterol in yeast. In plants, squalene gives rise to a number of pharmacologically active triterpenoids, such as β-amyrin and lupeol. Tetraterpenes (C_{40}) are produced through condensation of two molecules of GGPP, yielding phytoene, the precursor to the carotenoids, such as lycopene and β-carotene. Polyterpenes such as natural rubber (cis-polyisoprene) comprise thousands of isoprene units.

Alkaloids

In the broadest sense, alkaloids are defined as low-molecular-weight metabolites containing heterocyclic (true alkaloids) or exocyclic (protoalkaloids, amines, and polyamines) nitrogen atoms. Approximately 20,000 natural alkaloids are known, many of which exhibit analgesic (morphine), stimulant (caffeine and ephedrine), psychotropic (mescaline and cocaine), antibacterial (sanguinarine), anticancer (vinblastine and vincristine), antitussive (codeine), anti-inflammatory

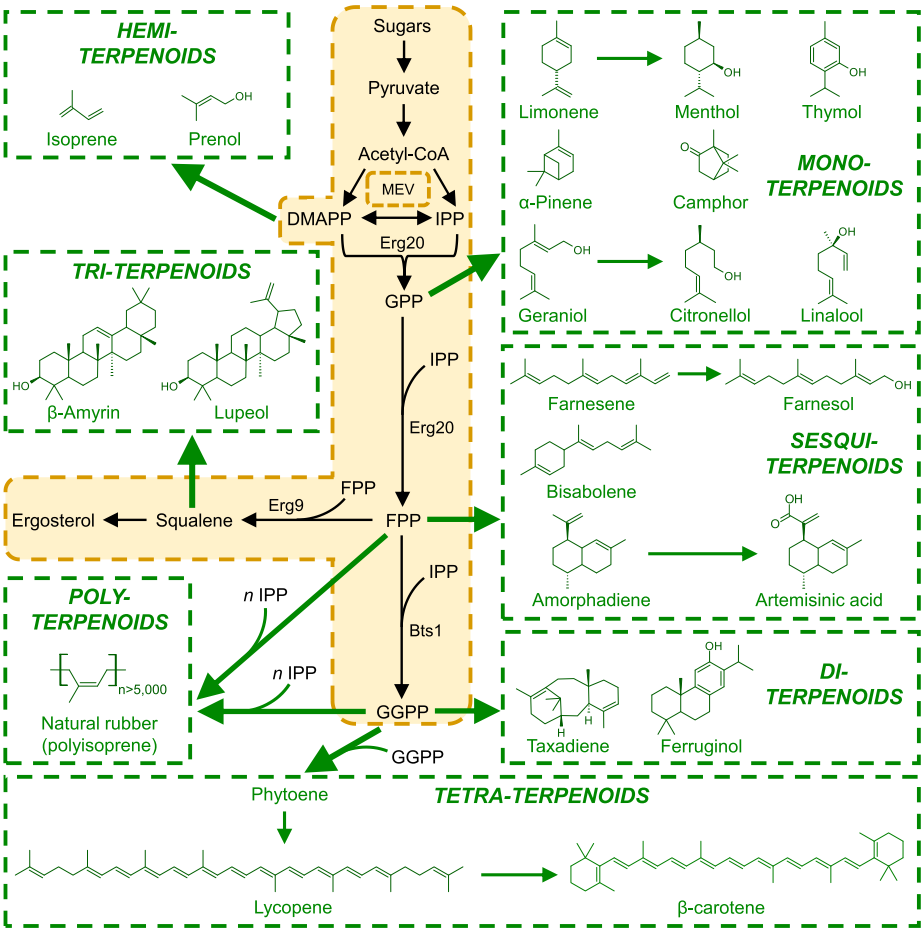


Figure 1. Interfacing plant terpenoid secondary metabolic pathways with microbial metabolism. Plant secondary metabolic reactions and pathways (green) are shown linked to core microbial metabolism (beige shading; black font). *S. cerevisiae* is shown as the prospective host species. Key yeast enzymes discussed in the main text are shown. Abbreviations: Bts1, geranylgeranyl diphosphate synthase; DMAPP, dimethylallyl pyrophosphate; Erg9, squalene synthase; Erg20, farnesyl pyrophosphate synthetase; FPP, farnesyl pyrophosphate; GPP, geranyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; IPP, isopentenyl pyrophosphate; MEV, mevalonate pathway.

(berberine), antispasmodic (papaverine), or antimalarial (quinine) activities.

Phe, Tyr, and their derivatives (e.g. phenethylamine, tyramine, and dopamine) are the source of a tremendous number of alkaloids, including the benzylisoquinoline alkaloids (BIAs) and the phenethylisoquinoline alkaloids (Fig. 2). BIAs are a large class of roughly 2,500 metabolites that includes berberine, noscapine, sanguinarine, and morphine. These important medicines are derived from the condensation of dopamine and 4-hydroxyphenylacetaldehyde, both of which are synthesized from Tyr. Dopamine can also condense with derivatives of Phe (4-hydroxydihydrocinnamaldehyde), yielding the phenethylisoquinoline class of alkaloids. Phe and Tyr form the basis of some simpler protoalkaloids and catecholamines, including modified amphetamines (ephedrine and cathinone), dopamine, mescaline, and adrenaline. Owing to the ubiquity of the indole group in nature, Trp also forms the basis of many important alkaloids, such as simple indoles, β-carbolines (serotonin and harmine), and the monoterpenoid indole alkaloids (MIAs).

MIAs, derived from the condensation of the Trp analog tryptamine and a monoterpenoid (secologanin), are one of the largest and most complex classes of alkaloids, giving rise to more than 3,000 structures. The Trp precursor anthranilate also serves as a precursor to several alkaloid subclasses, including the quinazolines. Beyond the aromatic amino acids, Arg and Orn form the basis of the tropane, pyrrolidine, pyrrolizidine, and pyridine alkaloids, whereas Lys is the precursor to the piperidines and quinolizidines. Nucleosides also form the basis of some alkaloids, such as caffeine and theobromine.

Phenylpropanoids and Polyketides

The phenylpropanoids represent a class of more than 8,000 plant phenolics derived from Tyr and Phe via the phenylpropanoid pathway (Wu and Chappell, 2008). The name phenylpropanoid refers to the distinctive C_6-C_3 structure of metabolites within this pathway. The key phenylpropanoid branch point intermediate is *p*-coumaroyl-CoA (Fig. 3), which forms the basis of the

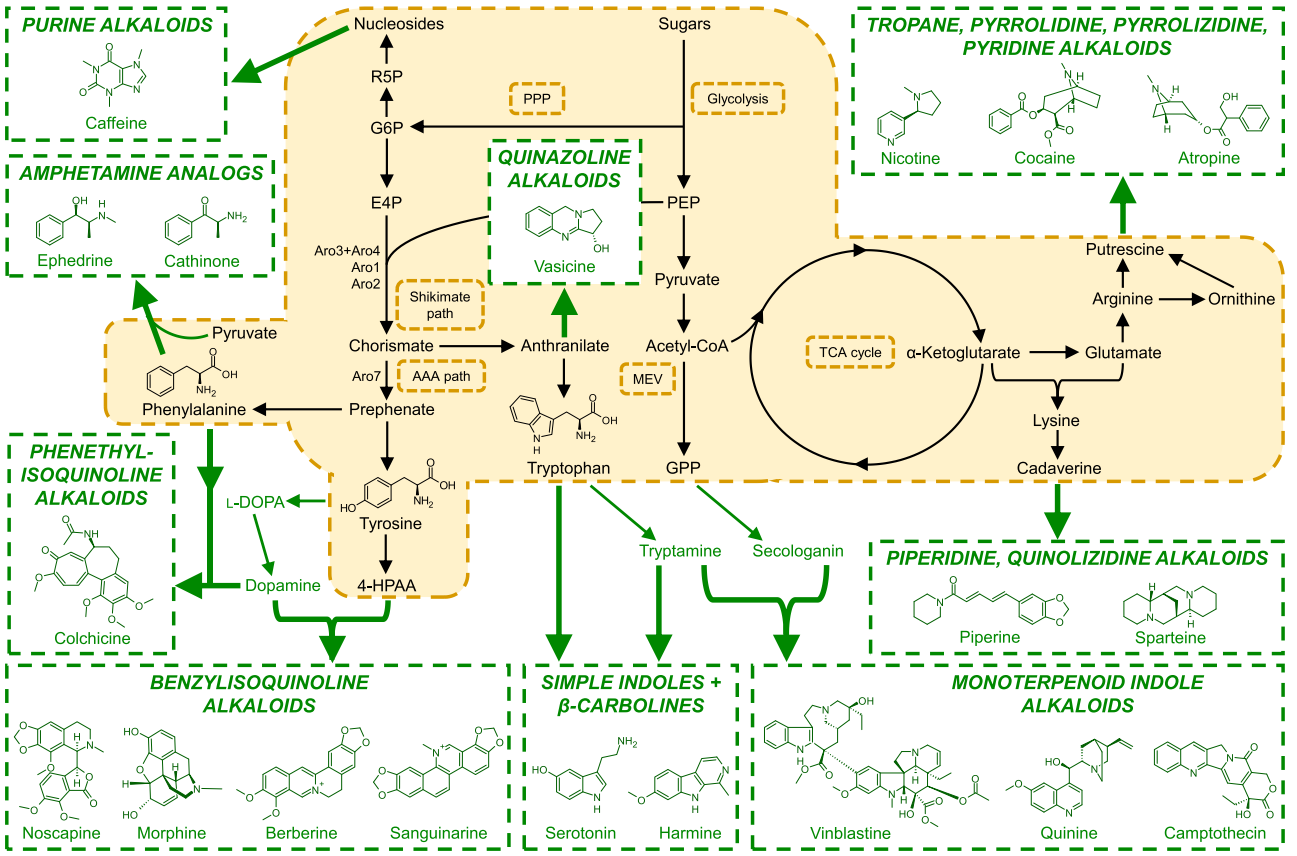


Figure 2. Interfacing plant alkaloid secondary metabolic pathways with microbial metabolism. *S. cerevisiae* is shown as the prospective host species. Refer to Figure 1 for abbreviations and color coding. Additional abbreviations: AAA path, aromatic amino acid pathway; Aro1, pentafunctional arom protein; Aro2, chorismate synthase and Flavin reductase; Aro3+Aro4, 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase isoenzymes; Aro7, chorismate mutase; E4P, erythrose 4-phosphate; 4-HPAA, 4-hydroxyphenylacetaldehyde; PPP, pentose phosphate pathway; PEP, phosphoenolpyruvate; R5P, ribose 5-phosphate; TCA cycle, tricarboxylic acid cycle.

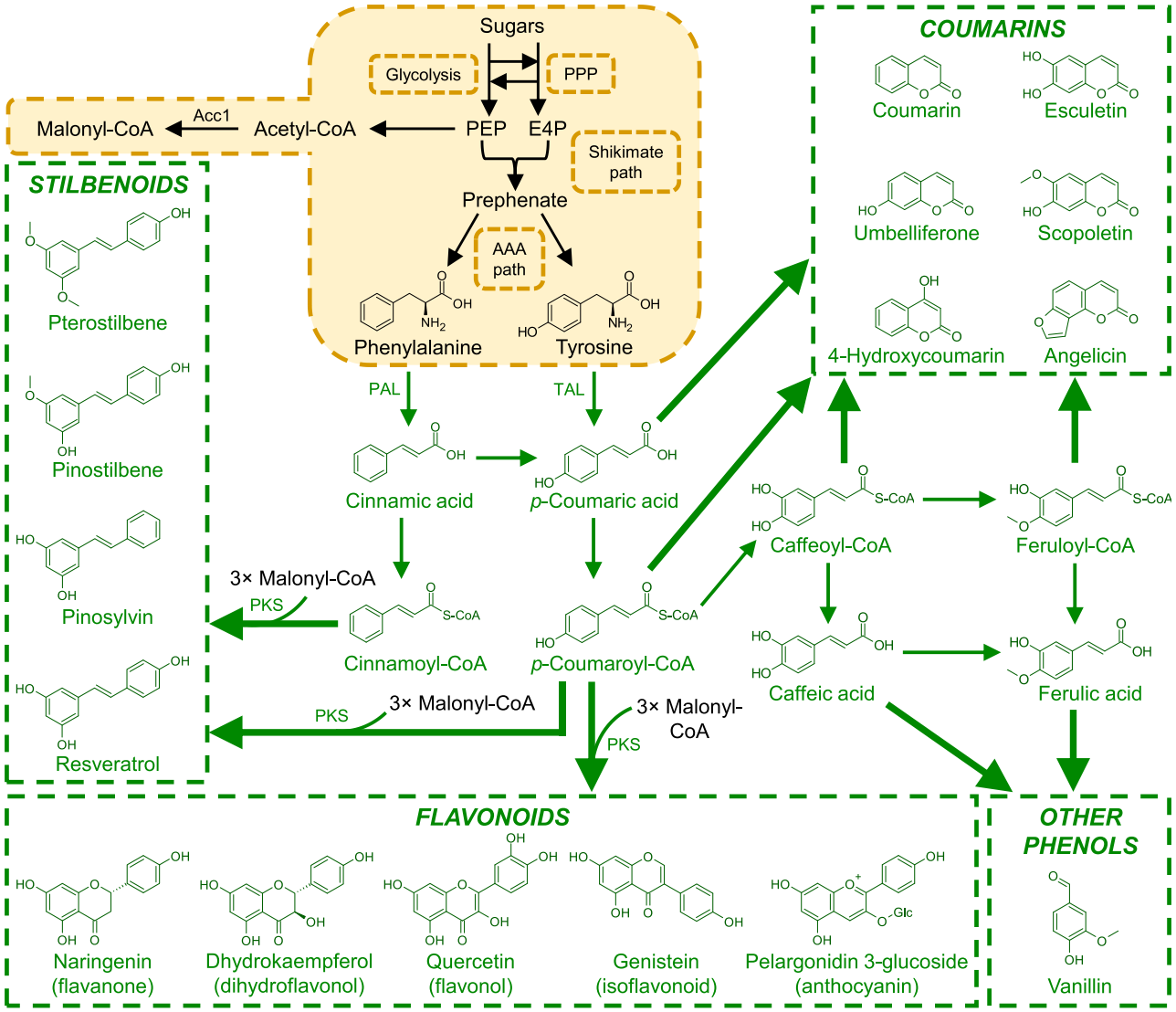


Figure 3. Interfacing plant phenylpropanoid and polyketide secondary metabolic pathways with microbial metabolism. A selection of natural plant pathways in a prospective *S. cerevisiae* host is shown. More extensive networks of natural and synthetic phenylpropanoid routes have been shown previously in Wang et al. (2015) and Zhao et al. (2015). Refer to Figures 1 and 2 for abbreviations and color coding. Additional abbreviations: Acc1, acetyl-CoA carboxylase; PAL, Phe ammonia-lyase; PKS, polyketide synthase; TAL, Tyr ammonia-lyase.

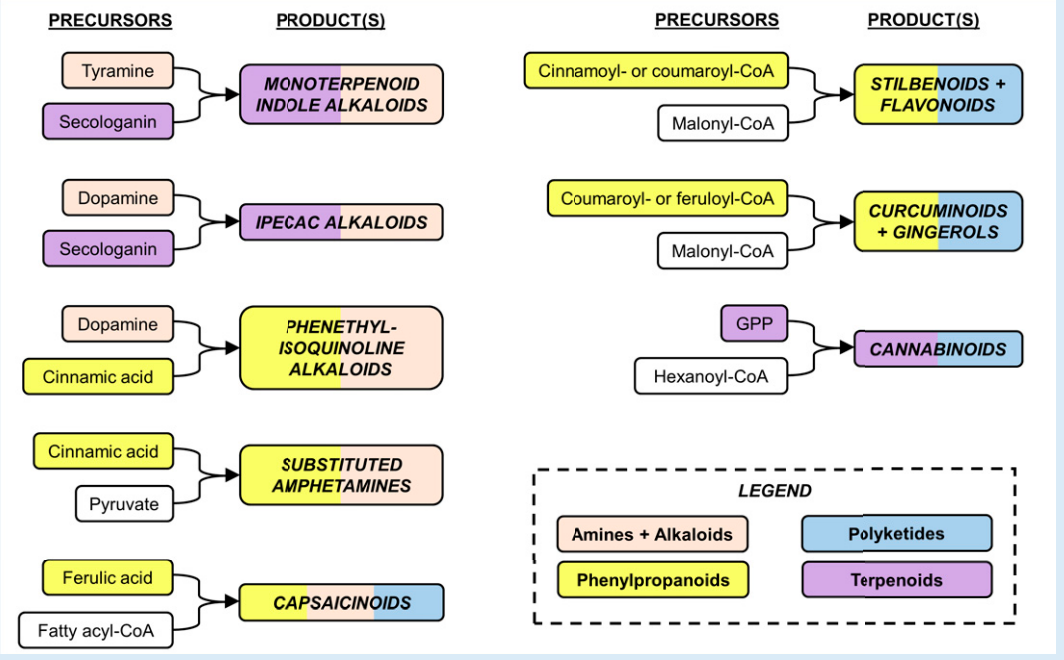
flavonoids, stilbenoids, coumarins, lignans, catechins, and aurones. In addition to the C_6-C_3 skeleton, the phenylpropanoid pathway also diverts at the level of cinnamic or ferulic acid to yield an array of C_6-C_1 benzoates, such as vanillin, benzaldehyde, and gallic acid (Vogt, 2010; Kallscheuer et al., 2018). Although the stilbenoids and flavonoids originate from the phenylpropanoid pathway, they are elongated by type III plant polyketide synthases (PKSs), underscoring the mixed biosynthetic nature of these specialized metabolites (Box 1). PKSs accept CoA-bound substrates (Yu et al., 2012), most often *p*-coumaroyl-CoA, although cinnamoyl- and feruloyl-CoA form the basis of some phenylpropanoid polyketides, such as pinosylvin and curcumin (Preisig-Müller et al., 1999; Kita et al., 2008). Once loaded with a starter molecule, malonyl-CoA moieties are incorporated into the growing polyketide chain.

Several different polyketide backbones can be produced, though the most heavily targeted for metabolic engineering are the naringenin chalcone and resveratrol scaffolds, which give rise to the respective flavonoid and stilbenoid subclasses (Lussier et al., 2013). These compounds are functionalized in downstream reactions including aromatic hydroxylation, NADP (NADPH)-dependent reduction, *O*-methylation, and glycosylation. The flavonoids alone encompass more than 6,000 natural products, including chalcones, aurones, catechins, flavanones, flavanols, isoflavonoids, and anthocyanins (Yu et al., 2012). In contrast to the

BOX 1. Metabolites of mixed biosynthetic origin

A number of important plant natural products are derived from mixed biosynthetic origins, obscuring the boundaries between secondary metabolite classes (Box 1 Figure). Within the alkaloid class, MIAs arise through condensation of the tryptophan analog tryptamine and a secoiridoid monoterpene (secologanin). Secologanin can also condense with dopamine, giving rise to emetine and cephaeline, the active alkaloids of the emetic drug ipecac. Nicotine is formed through coupling of two heterocyclic rings of distinct biosynthetic origin. Whereas the pyrrolidine ring derives from ornithine via the tropane alkaloid pathway (Figure 1), the pyridine ring is formed from niacin, itself synthesized from aspartate. Several other alkaloids are formed from products of the phenylpropanoid pathway, such as substituted amphetamines through condensation of pyruvate and benzoic acid, a C_6-C_1 analog of cinnamic acid (Hagel et al., 2012). Modified amphetamines are also called phenylpropylamino alkaloids to reflect their dual biosynthetic origin. Similarly, the autumnaline scaffold of the phenethylisoquinoline alkaloids is derived through condensation of dopamine and 4-hydroxydihydrocinnamaldehyde, another cinnamic acid derivative. The capsaicinoids produced by chili peppers have a unique tripartite classification, as they are exocyclic alkaloids that arise in part from the phenylpropanoid pathway and polyketide synthesis.

Specifically, coupling of ferulic-acid-derived vanillylamine and a fatty acyl-CoA (8-methyl-6-nonenoyl CoA) derived from valine or leucine gives rise to the capsaicin scaffold. Many other functionalized phenylpropanoids, notably the flavonoids and stilbenoids, are also polyketides involving chain elongation by malonyl-CoA (Figure 3). The curcuminoids and gingerols, responsible for the color and pungency of turmeric and ginger, respectively, are two additional subclasses comprising phenylpropanoid CoA esters linked by a polyketide synthase. Aromatic plant secondary metabolites are also frequently prenylated, which requires a terpenoid donor (Vickery et al., 2016). One such class of natural products is the cannabinoids, of which more than 100 distinct compounds are produced by the cannabis plant (Ahmed et al., 2015). A polyketide synthase catalyzes the decarboxylative condensation of hexanoyl-CoA with three molar equivalents of malonyl-CoA to generate olivetolic acid, which is then prenylated by GPP, generating cannabigerolic acid (CBGA). CBGA is the precursor to tetrahydrocannabinol (THC), cannabidiol, and their derivatives. These bioactive natural products have the potential to treat various medical conditions, including cancer, anorexia, epilepsy, and Alzheimer's and Huntington's diseases (Aizpurua-Olaizola et al., 2016).



flavonoids and stilbenoids, coumarins are not polyketides, as their lactone ring structure is derived from hydroxylation, isomerization, and lactonization of phenolic acids (cinnamic, *p*-coumaric, caffeic, or ferulic acid) or the corresponding phenolic acyl-CoA esters (Kai et al., 2008; Yao et al., 2017).

PIONEERING STUDIES IN THE MICROBIAL SYNTHESIS OF PLANT METABOLITES

The most famous application of synthetic biology for the synthesis of valuable plant products is semisynthetic artemisinin, an antimalarial terpenoid natively produced by sweet wormwood (*Artemisia annua*). Over a period of more than a decade, artemisinic acid production has reached titers of 25 g/L in yeast (Paddon et al., 2013), whereas *Escherichia coli* has been engineered to produce 27 g/L of the amorphadiene precursor (Tsuruta et al., 2009). The yeast artemisinic acid production strain has been repurposed as a sesquiterpenoid platform, facilitating industrial-scale production of more than 130 g/L farnesene (Meadows et al., 2016). Paclitaxel is another terpenoid medicine and microbes have been engineered to produce up to 1 g/L of the taxadiene scaffold (Ajikumar et al., 2010; Ding et al., 2014; Zhou et al., 2015). Other notable terpenoid pathway reconstructions include limonene, pinene, geraniol, eucalyptol, humulene, β-carophyllene, patchoulol, santalene, and bisabolene (Table 1).

Because geraniol forms half of the MIA scaffold, strategies successfully used to produce monoterpenoids were exploited to synthesize MIA intermediates in yeast, such as nepetalactol (Campbell et al., 2016; Billingsley et al., 2017) and strictosidine (Brown et al., 2015). These efforts have yet to be extended to the de novo synthesis of functionalized MIAs in a microbial system, although impressive partial pathway reconstructions have been reported (Qu et al., 2015). The BIA class has also recently experienced several breakthrough microbial pathway reconstitutions. As a result of successes in resolving upstream pathways involving conversion of Tyr to dopamine, de novo formation of (*S*)-reticuline was demonstrated for the first time (Nakagawa et al., 2011; DeLoache et al., 2015). This pathway was later extended for de novo biosynthesis of morphinan alkaloids in *E. coli* and yeast (Galanie et al., 2015; Nakagawa et al., 2016), as well as noscapine in yeast (Li et al., 2018). Titers of (*S*)-reticuline have recently been increased to 160 mg/L in *E. coli* (Matsumura et al., 2018). Stilbenoids have been the target of several microbial synthesis efforts and the de novo titer of resveratrol has reached 5 g/L in yeast (Katz et al., 2013; Li et al., 2016b). Other stilbenoids produced in microbes include pinostilbene, pterostilbene, pinosylvin, and piceatannol (Table 1). Similarly, the branch point flavonoid naringenin has been produced from glucose at titers above 200 mg/L (Lehka et al., 2017). The flavonoid pathway has been diversified in *E. coli* and yeast to yield the dihydrochalcone phloretin and its derivatives, the flavanone liquiritigenin, and the flavonols kaempferol, quercetin, and fisetin, among others (Table 1). This pathway was extended to produce anthocyanin pigments using *E. coli* polycultures (Jones et al., 2017) and a single engineered yeast strain (Eichenberger et al., 2018; Levisson et al., 2018).

Not all natural product syntheses involve straightforward maximization of product titer, because some applications require careful tuning of product concentrations. One such study involved combinatorial engineering of industrial brewing yeasts (*S. cerevisiae*) to generate strains with a diverse range of geraniol and linalool production profiles, culminating in the formulation of beers with a more desirable hops flavor compared with commercial varieties (Denby et al., 2018). Studies such as these highlight the wide range of applications afforded through the manipulation of plant metabolic pathways in microbial systems.

ELUCIDATION OF PLANT METABOLIC PATHWAYS

In Planta

Traditionally, the elucidation of metabolic pathways in source plants has been the driving force and often limiting factor of microbial biosynthesis. Owing to the complexity of plant metabolism, gaps in pathways are common, particularly within downstream reactions mediating the formation of functionalized products. Pathway gaps in the context of microbial synthesis refer to a lack of enzyme identification, as the biochemical nature of metabolic transformations is typically known.

To bypass gaps in enzyme elucidation, pathway intermediates are often supplied to cells exogenously, granted they are available and an uptake system exists. Although enzyme gaps have plagued many natural product pathways, several important pathways have been completed. While it was known that production of the nepetalactol backbone within the MIA pathway involved reductive cyclization of 10-oxogeranial, the enzyme mediating this transformation was finally discovered in 2012 (Geu-Flores et al., 2012). Critical to this success was the search of source plant transcriptomes for redox enzymes coexpressed with MIA pathway transcripts. Other key enzymes within the early MIA pathway were discovered in 2014 (Miettinen et al., 2014), prompting the synthesis of the key branch point MIA strictosidine in yeast soon thereafter (Brown et al., 2015).

The MIA pathway leading to vinblastine was fully elucidated (Caputi et al., 2018); thus, it is only a matter of time before the de novo synthesis of this potent anticancer medicine is achieved in a microbial system. Similarly, the final enzymes involved in the formation of substituted amphetamines, such as ephedrine and pseudoephedrine (Hagel et al., 2012), were also identified (Morris et al., 2018), providing rationale for their synthesis in a microbial system. The opium poppy

Table 1. *Selection of de novo plant secondary pathway reconstructions in microbial systems*

ND, no data available; NR, titer not reported.

Metabolite class	Metabolite	Titer (mg/L)		Reference(s)
		S. cerevisiae	*E. coli*	
PRECURSORS	Tyr	1,930[a]	55,000	(Patnaik et al., 2008)
	p-coumaric acid	1,930	2,510	(Rodriguez et al., 2015; Jones et al., 2017)
	dopamine	24	2,150	(Nakagawa et al., 2014; DeLoache et al., 2015)
TERPENOIDS	*Monoterpenoids*			
	geraniol	1,680	2,000	(Liu et al., 2016; Jiang et al., 2017)
	pinene	ND	970	(Yang et al., 2013)
	limonene	0.49	2,700	(Willrodt et al., 2014; Jongedijk et al., 2015)
	eucalyptol	400	653	(Ignea et al., 2011; Mendez-Perez et al., 2017)
	linalool	0.75	505	(Mendez-Perez et al., 2017; Camesasca et al., 2018)
	Sesquiterpenoids			
	β-farnesene	130,000	8,700	(Meadows et al., 2016; You et al., 2017)
	bisabolene	5,200	912	(Peralta-Yahya et al., 2011; Özaydin et al., 2013)
	α-humulene	1,300	ND	(Zhang et al., 2018b)
	β-caryophyllene	ND	1,520	(Yang et al., 2016)
	amorphadiene	37,000	27,400	(Tsuruta et al., 2009; Westfall et al., 2012)
	artemisinic acid	25,000	ND	(Paddon et al., 2013)
	santalene	163	ND	(Tippmann et al., 2016)
	patchoulol	40.9	ND	(Albertsen et al., 2011)
	Diterpenoids			
	jolkinol C	800	ND	(Wong et al., 2018)
	taxadiene	72.8	1,020	(Ajikumar et al., 2010; Ding et al., 2014)
	Triterpenoids			
	β-amyrin	108.1	NR	(Takemura et al., 2017; Zhu et al., 2018)
	Tetraterpenoids			
	β-carotene	162.1	3,200	(Yang and Guo, 2014; Wang et al., 2016)
	lycopene	1,650	3,520	(Sun et al., 2014; Chen et al., 2016)
ALKALOIDS	*Benzylisoquinoline alkaloids*			
	(*S*)-reticuline	0.082	160	(DeLoache et al., 2015; Matsumura et al., 2018)
	thebaine	0.0064	2.1	(Galanie et al., 2015; Nakagawa et al., 2016)
	hydrocodone	0.0003	0.36	(Galanie et al., 2015; Nakagawa et al., 2016)
	noscapine	2.2	ND	(Li et al., 2018)
	Monoterpene indole alkaloids			
	strictosidine	0.5	ND	(Brown et al., 2015)
PHENYLPROPANOIDS	*Stilbenoids*			
	resveratrol	>5,000	51.8	(Katz et al., 2013; Yang et al., 2018)
	pinostilbene	5.52	~2.5	(Kang et al., 2014; Li et al., 2016b)
	pterostilbene	34.93	33.6	(Li et al., 2016b; Heo et al., 2017)
	pinosylvin	130.02	281	(Katz et al., 2008; Wu et al., 2017)
	Flavonoids			
	naringenin	>200	103.8	(Lehka et al., 2017; Yang et al., 2018)
	scutellarin	108	ND	(Liu et al., 2018)
	genistein	7.7	ND	(Trantas et al., 2009)
	pinocembrin	2.6	525.8	(Wu et al., 2016b; Eichenberger et al., 2017)
	quercetin	20.38	ND	(Rodriguez et al., 2017)

(*Table continues on following page.*)

Table 1. (*Continued from previous page.*)

Metabolite class	Metabolite	Titer (mg/L)		Reference(s)
		S. cerevisiae	*E. coli*	
	kaempferol	66.29	57[b]	(Yang et al., 2014; Duan et al., 2017)
	dihydrokaempferol	44	ND	(Levisson et al., 2018)
	pelargonidin-3-*O*-glucoside	0.02	9.5	(Jones et al., 2017; Levisson et al., 2018)
	phloretin	42.7	ND	(Eichenberger et al., 2017)
Coumarins				
	umbelliferone	ND	66.1	(Yang et al., 2015a)
	esculetin	ND	61.4	(Yang et al., 2015a)
	4-hydroxycoumarin	ND	483.1	(Lin et al., 2013)

[a]Titer reported for the downstream metabolite *p*-coumaric acid. [b]Titer reported for kaempferol 3-*O*-rhamnoside.

(*Papaver somniferum*) enzyme responsible for the stereochemical inversion of (*S*)- to (*R*)-reticuline eluded identification for many years, limiting morphine pathway reconstructions to upstream [Glc to (*S*)-reticuline] and downstream [(*R*)-reticuline to morphine] modules. Eventual identification of the fusion enzyme by three research groups led to the reconstruction of the de novo morphinan pathway within a microbial host (Galanie et al., 2015). Candidate epimerase genes were identified using transcriptome database mining, and candidates were silenced in opium poppy plants sequenced from mutants that accumulate (*S*)-reticuline, or cloned in *S. cerevisiae* for functional analysis (Farrow et al., 2015; Galanie et al., 2015; Winzer et al., 2015). While this sequence of events permeates the field of microbial synthesis, in which pathway elucidation in planta precedes pathway reconstruction in microbes, a paradigm has arisen in response to this laborious and time-consuming process.

In Microbes

Due to the declining cost of DNA synthesis, rapidly advancing bioinformatics tools, and expanding omics databases, it has become possible to resolve single and multienzyme gaps within a heterologous microbial host. Using this synthetic biology approach, libraries of candidate gap-filling enzymes are compiled by querying plant transcriptome databases, such as 1000 Plants (Matasci et al., 2014) or PhytoMetaSyn (Xiao et al., 2013) Projects. The corresponding genes are then codon-optimized, synthesized, and expressed in a laboratory microbe. This workflow facilitates the resolution of enzyme gaps in plant metabolic pathways without having to obtain physical plant material or cDNAs (Narcross et al., 2016a). In this regard, synthetic biologists do not need to wait for gap-filling enzymes to be isolated and characterized from natural sources to reconstitute target pathways in a microbial species. One of the groups involved in identifying the aforementioned reticuline epimerase gene employed this strategy by querying plant transcriptome databases and integrating synthetic genes into a yeast strain harboring a downstream module for thebaine synthesis (Galanie et al., 2015). An ambitious combinatorial variation of this technique was attempted to fill a seven-enzyme gap within the vinblastine MIA pathway (Casini et al., 2018). While Casini et al. (2018) were unable to resolve this intricate pathway, they succeeded in building 74 strains, each possessing a distinct combination of the seven gene candidates. This study illustrates the immense potential of synthetic biology for elucidating and reconstructing complex biochemical pathways in microbial systems.

HOST SELECTION

Host selection is a fundamental facet of microbial synthesis and a key determinant of pathway

performance. Because it is virtually impossible to forecast the ideal host for synthesizing a target metabolite, numerous factors must be weighed, including access to a complete genome sequence and the availability of genetic tools. Precursor availability is perhaps the most important criterion of host selection, as an abundant precursor pool, such as acetyl-CoA or shikimate, sidesteps the need to rewire core metabolism. Host selection is further complicated by the existence of phenotypically distinct strains within a given species, for instance the *E. coli* K and B lineages or the S288C and CEN.PK strains of *S. cerevisiae*. In a dramatic instance, use of *E. coli* DH1 improved oxidation of amorphadiene by a factor of 1000 relative to related strains (Chang et al., 2007). Screening of *E. coli* lineages for polyketide synthesis has revealed not only strain-specific differences in baseline polyketide production but also differences in response to the same genetic manipulations (Yang et al., 2018). Due to differences in amino acid biosynthesis between *S. cerevisiae* (Canelas et al., 2010), production of vanillin-β-glucoside was significantly higher in S288C than in CEN.PK (Strucko et al., 2015). Similar yeast-specific differences were observed for the synthesis of the polyketide triacetic acid lactone (Saunders et al., 2015). These studies highlight the often overlooked task of screening strains to identify optimal producers prior to comprehensively engineering a production strain.

Workhorses and Specialized Hosts

The overwhelming majority of microbial syntheses have employed *E. coli* or *S. cerevisiae* owing to their ease of genetic and metabolic manipulation, a comprehensive understanding of their genetics, metabolism, and physiology, and their highly active central metabolic pathways. Several critical differences between these industry-proven hosts inform many microbial synthesis campaigns (Box 2).

Recently, the diversity of microbial hosts exploited for the synthesis of plant natural products has expanded dramatically. Some microorganisms naturally synthesize aromatic amino acids at levels well above those of *E. coli* and *S. cerevisiae*, indicating a major potential of these hosts for the production of alkaloids and phenylpropanoids. Of particular importance is *Corynebacterium glutamicum*, an industrial bacterium with a tremendous capacity to synthesize glucose and Lys, as well as aromatic amino acids (Azuma et al., 1993; Ikeda et al., 1993). *Lactococcus lactis* is another industry-proven bacterium with a demonstrated capacity to synthesize plant terpenoids and phenylpropanoids

BOX 2. *E. coli* versus *S. cerevisiae*

Most microbial synthesis projects start with the question: *E. coli* or *S. cerevisiae* Yeast alcohol production exemplifies the most ancient industrial bioprocess and both hosts have been exploited extensively in industrial biotechnology. Although many plant CYPs and CPRs can be functionally expressed in bacteria, often following extensive engineering of N-terminal membrane domains, CYPs are typically functional in yeast without modification. Because yeast produces only three native CYPs, interference from heterologous CYP and CPR proteins is generally not problematic. Therefore, we surmise that yeast is the superior host to support the biosynthesis of highly functionalized plant secondary metabolites, particularly those arising from multi-CYP pathways. The occurrence of eight CYP-catalyzed reactions in the downstream taxane pathway precludes the use of bacterial systems for producing these important products. *E. coli* has been engineered to synthesize the highly functionalized morphinan BIA hydrocodone (Nakagawa et al., 2016), yet the full *de novo* pathway was split between four engineered strains. The same pathway has been reconstructed within a single *S. cerevisiae* strain (Galanie et al., 2015), albeit at lower titers, again highlighting the potential advantages of eukaryotic hosts for synthesizing complex plant secondary metabolites. Analogously, *de novo* anthocyanin synthesis in *E. coli* employed four engineered strains (Jones et al., 2017), which again contrasts strategies using *S. cerevisiae* in which a single strain hosts the full anthocyanin pathway (Eichenberger et al., 2018; Levisson et al., 2018). Although yeast is clearly favored for harboring downstream pathways, precursor titers are often higher in *E. coli* (e.g., for production of tyrosine, *p*-coumaric acid, dopamine, reticuline, and taxadiene; Table 1). For instance, *E. coli* has been engineered to produce 1 g/L of taxadiene (Ajikumar et al., 2010), which contrasts yeast titers of only 72.8 mg/L (Ding et al., 2014). This disparity in precursor production and downstream functionalization points to co-culture strategies in which natural product pathways are divided between *E. coli* and *S. cerevisiae* hosts. This approach proved highly effective for the synthesis of functionalized taxanes,in which the taxadiene precursor was secreted by *E. coli* and imported and derivatized by *S. cerevisiae* harboring multiple CYP enzymes (Zhou et al., 2015).

(Gaspar et al., 2013; Dudnik et al., 2018). The yeast *Scheffersomyces stipitis* has gained interest for its ability to use C_5 sugars, suggesting that this organism has a more active pentose phosphate pathway than does *S. cerevisiae*. Indeed, the highest shikimate titer reported to date in yeast was achieved using *S. stipites* (Gao et al., 2017).

Within the polyketide class of natural products, bacteria from the genus *Streptomyces* are major natural producers, providing rationale for diverting these native pathways to plant-derived polyketides (Baltz, 2010). The oleaginous yeast *Yarrowia lipolytica* has also been demonstrated to be a promising host for terpenoids, polyketides, and other compounds derived from acetyl-CoA (Abdel-Mawgoud et al., 2018). Other hosts are desirable for superior growth characteristics, such as *Pichia pastoris*, a yeast that attains higher cell densities than *S. cerevisiae* (Wriessnegger et al., 2014).

RECONSTITUTION AND OPTIMIZATION OF PLANT PATHWAYS IN MICROBIAL SYSTEMS

Pathway Assembly and Delivery

Pathway assembly refers to the design and organization of genetic parts required to reconstruct heterologous pathways in microbes. This endeavor begins by selecting and synthesizing gene variants responsible for mediating the synthesis of the target product. The selected candidates are placed within transcriptional units containing a promoter and terminator. Traditionally, heterologous genes and metabolic pathways have been delivered and expressed from plasmids. While this technique has certain merits, such as facilitating the rapid screening of prospective hosts (Casini et al., 2018) and easily assessing the performance of pathway modules (Fossati et al., 2014), access to efficient genome editing tools has led to the adoption of chromosomal expression systems (Horwitz et al., 2015; Jakočiūnas et al., 2015). Furthermore, plasmid-borne expression leads to significant variability in gene expression and copy number between cells (Ryan et al., 2014).

With respect to transcriptional control, most pathways are assembled by placing all genes under control of constitutive promoters. For instance, amorphadiene and artemisinic acid production strains were constructed using Gal induction, which was later converted to constitutive expression without affecting production or fitness (Westfall et al., 2012; Paddon et al., 2013). Because even "constitutive" promoters exhibit distinct transcriptional profiles, which vary based on carbon source or medium formulation (Reider Apel et al., 2017), major efforts have focused on comprehensively characterizing both promoter and terminator elements (Yamanishi et al., 2013; Lee et al., 2015). These toolkits facilitate the exploration of vast pathway design spaces comprised of a staggering number of possible gene and expression combinations.

Product titer, rate, and yield following initial pathway reconstitution are often very poor, necessitating many design-build-test engineering cycles to reach commercially viable levels. Historically, this process lasts six to eight years and costs more than $50 million (Nielsen and Keasling, 2016). These metrics are declining at a dramatic rate as a result of the standardization of synthetic biology and the advent of high-throughput strain engineering facilities ("biofoundries"), which aim to miniaturize and automate strain construction workflows (Chao et al., 2017; Casini et al., 2018).

Expression of Plant Genes in Microbial Species

The functional expression of plant genes in microbial species poses technical challenges. Many plant enzymes possess localization signals for targeting to specific organelles, such as the chloroplast. Implementation of these plant genes in microbial hosts typically leads to poor gene expression or insolubility of the corresponding protein (Williams et al., 1998). Most terpene synthases are plastid-localized and thus N-terminal truncation is an effective strategy for improving activity (Alonso-Gutierrez et al., 2013; Zhao et al., 2016). Production of BIAs is also improved through N-terminal truncation of norcoclaurine synthase (Li et al., 2016a). While software tools exist for predicting optimal signal peptide cleavage sites (Petersen et al., 2011), assessing various-sized truncations affords a range of improvements (Nishihachijo et al., 2014; Jiang et al., 2017).

Perhaps the most challenging facet of synthesizing plant-derived metabolites in microbial hosts is the functional expression of cytochrome P450 (CYP) enzymes. This obstacle provides much of the rationale for using eukaryotic expression systems over bacteria (Box 2). CYPs are membrane-bound enzymes that carry out many of the complex functionalizations in secondary metabolic pathways, including hydroxylations, double bond epoxidations, and dealkylations (Meunier et al., 2004). Because CYPs mediate oxidation reactions, catalysis is coupled to reduction of NADPH, which in turn requires a cytochrome P450 reductase (CPR) partner to shuttle electrons between NADPH and CYP. Additional components can also be involved, such as cytochrome b_5 and a cytochrome b_5 reductase (Porter, 2002; Paddon et al., 2013; Li et al., 2016b).

Some species of plant contain hundreds of CYPs and multiple CPRs, leading to immense challenges in optimizing heterologous CYP-CPR pairings. Although yeast contains a native CPR, most plant CYPs exhibit greater activity when paired with CPRs from the same plant species (Fossati et al., 2014). Production of highly functionalized secondary metabolites, such as paclitaxel and hydrocodone, involves numerous CYP-catalyzed reactions, requiring the coordinated expression of multiple CPR genes. To overcome this hurdle, most microbial synthesis studies employ a "one-size-fits-all" strategy by pairing multiple CYPs with a single CPR. CYP and CPR expression must be carefully balanced to ensure efficient shuttling of electrons and

avoid the production of toxic reactive oxygen species. Optimization of CYP-CPR pairing was paramount to the success of the semisynthetic artemisinin process (Paddon et al., 2013) and enabled production of 500 mg/L oxygenated taxadiene in *E. coli* (Biggs et al., 2016).

The most efficient natural CYP is a primitive bacterial CYP-CPR fusion from *Bacillus megaterium* (Munro et al., 2002). This architecture has been exploited for the expression of plant CYPs in *E. coli*, in which the N-terminal membrane-binding domain is cleaved or modified for more efficient insertion within bacterial membranes (Hausjell et al., 2018). Conversely, it has been shown that a direct CYP-CPR fusion in *E. coli* was less active than carefully balanced expression of both components for oxygenated taxadiene synthesis (Biggs et al., 2016). Biggs et al. (2016) suggest that because plant CYPs and CPRs do not interact in a 1:1 ratio, a direct fusion approach is not appropriate.

Precursor Supply

Engineering microbial systems for the production of phenylpropanoids and most alkaloids demands an abundant supply of aromatic amino acid precursors (Figs. 2 and 3). Tremendous strides have been made in engineering platform strains producing high levels of Tyr, Phe, Trp, and their derivatives (Table 1).

Deregulating host precursor pathways is regarded as the chief hurdle to the production of heterologous metabolites (Nielsen and Keasling, 2016). The yeast shikimate and aromatic amino acid pathways are subject to multiple layers of transcriptional and posttranscriptional control. The entry point to the shikimate pathway involving condensation of E4P and PEP is catalyzed by 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase, for which two isoenzymes exist in yeast (Aro3 and Aro4). Allosteric inhibition by Tyr can be overcome in both enzymes using analogous mutations ($Aro3^{K222L}$ and $Aro4^{K229L}$; Luttik et al., 2008; Brückner et al., 2018). Similarly, chorismate mutase (Aro7) is feedback-inhibited by Tyr, prompting discovery of the $Aro7^{G141S}$ allele for deregulating Tyr biosynthesis (Luttik et al., 2008). Implementation of $Aro4^{K229L}$ and $Aro7^{G141S}$ in the same host leads to a 200-fold increase in extracellular levels of aromatic compounds.

The terpenoid branch of secondary metabolism has also been the focal point of engineering precursor supply. The rate-limiting step of the mevalonate pathway catalyzed by HMG-CoA reductase (HMGR) can be improved by implementing a truncated mutant (tHMGR). Owing to the importance of this enzymatic step, studies achieving high-level terpenoid or MIA production have incorporated up to four gene copies of *tHMGR* (Paddon et al., 2013; Brown et al., 2015).

The production of GPP-derived monoterpenoids poses a unique engineering challenge pertaining to the dual nature of the yeast Erg20 protein. This enzyme catalyzes the synthesis of GPP from IPP and dimethylallyl pyrophosphate, as well as the subsequent condensation of GPP and an additional unit of IPP to give FPP (Fig. 1). Owing to the central role of FPP in ergosterol biosynthesis, Erg20 is an essential enzyme, signifying that its FPP synthase activity cannot be abolished. Instead, Erg20 variants with reduced GPP-binding properties have been described (Chambon et al., 1990) and exploited for the production of various monoterpenoids and MIAs (Brown et al., 2015; Campbell et al., 2016). Relatedly, the essential squalene synthase enzyme (Erg9) generates squalene from two FPP units, thus placing constraints on the production of all terpenoids deriving from FPP (Fig. 1).

The most fundamental means of circumventing host regulation is replacing native precursor pathways with a heterologous counterpart. This approach was used in early efforts to produce amorphadiene in *E. coli*, wherein the native MEP pathway was replaced by the yeast mevalonate pathway (Martin et al., 2003). Reconstructing the yeast pathway within a bacterial operon eliminates both *E. coli* and *S. cerevisiae* modes of regulation, facilitating constitutive production of terpenoids.

Acetyl-CoA plays a pivotal role in the biosynthesis of terpenoids, polyketides, phenylpropanoids, and cannabinoids (Figs. 1 and 3), and its overproduction has also been a target for improvement. In yeast, multiple acetyl-CoA synthesis pathways exist in different subcellular compartments that acetyl-CoA cannot directly cross (Strijbis and Distel, 2010). This provides options for strain engineering based on the pathway that is most suited for a particular heterologous product (van Rossum et al., 2016). Alternatively, heterologous cytosolic acetyl-CoA pathways are of great interest (Kozak et al., 2014a, 2014b, 2016; Zhang et al., 2015; Cardenas and Da Silva, 2016; Meadows et al., 2016; Rodriguez et al., 2016). The highest reported titer of a heterologous product derived from cytosolic acetyl-CoA is the synthesis of 130 g/L of the sesquiterpene farnesene under fermentative conditions (Meadows et al., 2016). This approach used multiple alternative cytosolic acetyl-CoA pathways to overproduce farnesene.

In prokaryotes, all routes to acetyl-CoA are equally accessible, reducing the necessity for alternative pathways, although they have been demonstrated (Wang et al., 2018). However, buildup of acetyl-CoA results in its conversion to acetate, which is an unproductive carbon sink. In *E. coli*, native acetate assimilation enzymes can be overexpressed to convert acetate back to acetyl-CoA, resulting in improved flavanone biosynthesis (Leonard et al., 2007; Zha et al., 2009). Gene knockouts have been identified that prevent acetate synthesis and improve heterologous production without reducing cellular fitness (Zha et al., 2009; Liu et al., 2017a). Eliminating pathway bottlenecks to improve flux through acetyl-CoA can also reduce acetate overflow (Li et al., 2015; King et al., 2017; Wang et al., 2018). Relatively little attention has been placed on the availability of free Coenzyme A to accept increased carbon flux. Supplementation of the Coenzyme A precursor

pantothenate can improve the yield of heterologous products requiring acetyl-CoA. Several studies have demonstrated that overexpression of the rate-limiting step of Coenzyme A synthesis, pantothenate kinase, improves product synthesis 2-fold (Fowler et al., 2009; Schadeweg and Boles, 2016; Liu et al., 2017b).

Phenylpropanoid, polyketide, and cannabinoid synthesis require malonyl-CoA, which is used for fatty acid biosynthesis in *E. coli* and *S. cerevisiae*. In these organisms, fatty acid synthesis is tightly regulated (Davis et al., 2000). Improving synthesis and reducing off-target consumption of malonyl-CoA are the major areas for overproduction. Acetyl-CoA is converted to malonyl-CoA by acetyl-CoA carboxylase (Acc), and improvements in this activity have been achieved by overexpression (Zha et al., 2009; Xu et al., 2011; Yang et al., 2018), ablating posttranslational modification sites (Choi and Da Silva, 2014; Shi et al., 2014; Chen et al., 2018), and improving the availability of its biotin cofactor (Leonard et al., 2007). Acc-independent malonyl-CoA synthesis has also been demonstrated (Leonard et al., 2008), though the pathway requires supplemented malonate. Reducing the diversion of malonyl-CoA to essential pathways is a challenge, because fatty acids are required for biomass production. In yeast, knockout of genes regulating fatty acid biosynthesis improved titers of malonyl-CoA-derived products (Zha et al., 2009; Chen et al., 2017), yet the strain was auxotrophic for inositol. More promisingly, biomass accumulation prior to conditional gene knockdown has been demonstrated to improve polyketide titers in *E. coli* (Yang et al., 2015b, 2018; Liang et al., 2016; Wu et al., 2016a).

As a ubiquitous cofactor and the source of reductant for plant CYPs, NADPH is an important engineering target for the synthesis of natural products. A proven strategy for enhancing NADPH availability is the inactivation of native NADPH-consuming reactions. A caveat of this approach pertains to the targeting of enzymes involved in central carbon metabolism, such as Glc-6-phosphate isomerase and phosphoenolpyruvate carboxylase in *E. coli* (Chemler et al., 2010) or Glc-6-phosphate dehydrogenase in *S. cerevisiae* (Gold et al., 2015). Glucose dehydrogenase is a promising candidate in both species (Asadollahi et al., 2009; Chemler et al., 2010). Inactivation of these central targets often has deleterious effects on strain fitness, including the generation of amino acid auxotrophy (Gold et al., 2015), demanding a delicate balancing of core pathways and sophisticated metabolic models for predicting combinatorial modifications (Chemler et al., 2010).

The counter approach of up-regulating NADPH-generating enzymes is a more viable avenue for engineering NADPH supply. In yeast, these enzymes include prephenate dehydrogenase (Tyr-1) and cytosolic aldehyde dehydrogenase (Li et al., 2018). Curiously, both overexpression and deletion of Glc-6-phosphate dehydrogenase have been shown to enhance formation of Tyr products (Gold et al., 2015; Li et al., 2018), underscoring the complexity of yeast central metabolism and redox homeostasis.

Improving Flux to Heterologous Pathways

Flux improvement involves iterative rounds of strain engineering with the aim of elevating product titer, rate, and yield. Efforts to improve flux through a target pathway begin with the identification of rate-limiting enzymatic conversions, which is diagnosed by monitoring levels of pathway intermediates. The most fundamental means of relieving metabolic bottlenecks is to increase levels of rate-limiting enzymes by increasing gene expression or copy number. In addition to high-level expression of the heterologous pathway, the entire eight-gene yeast pathway from acetyl-CoA to FPP was overexpressed in the industrial artemisinic acid strain (Paddon et al., 2013).

Poor pathway flux can also arise from the diversion of pathway intermediates to off-target routes, either by host activities or promiscuous plant enzymes. Because phenylpropanoid biosynthesis derives from Phe or Tyr, the yeast pathway for aromatic amino acid degradation (Ehrlich pathway) is typically inactivated through deletion of amino acid decarboxylase genes (*ARO10* and *PDC5*; Rodriguez et al., 2015). Yeast BIA biosynthesis involves a more intricate balancing of Tyr pathways, as the dopamine and 4-hydroxyphenylacetaldehyde precursors are derived from its biosynthesis and degradation, respectively (Narcross et al., 2016b). The physiological fate of 4-HPAA in yeast involves conversion to tyrosol or 4-hydroxyphenylacetate, which can be mediated by more than 20 candidate enzymes (Hazelwood et al., 2008; DeLoache et al., 2015). These enzymes have also been implicated in the irreversible reduction of aldehyde intermediates within the MIA pathway (Billingsley et al., 2017), suggesting that any pathway possessing carbonyl intermediates is subject to diversion by host activities. In a similar scenario, a yeast reductase was shown to divert *p*-coumaroyl-CoA within the phenylpropanoid pathway to an unwanted side product (Lehka et al., 2017).

Pathway flux can also be diverted to off-target routes by heterologous pathway enzymes. *O*- and *N*-methyltransferases within the core BIA pathway have been shown to exhibit a wide substrate range (Frick et al., 2001; Ounaroon et al., 2003; Fossati et al., 2014), which was circumvented by screening of plant homologs for variants exhibiting a more stringent substrate specificity (Narcross et al., 2016a). In addition to catalyzing the reductive cyclization of 10-oxogeranial, iridoid synthase within the MIA pathway was found to exhibit activity on other intermediates (Campbell et al., 2016). In this case, enzyme activity was higher on the unwanted substrates than the physiological substrate. Enzyme promiscuity may be avoided in cocultures, in which a pathway is split between strains such that promiscuous enzymes are sequestered from pathway intermediates (Nakagawa et al., 2014).

Loss of intermediates is also mitigated through enzyme fusion, as in the cases of geraniol synthase or patchoulol synthase through fusion to yeast farnesyl diphosphate synthase (Erg20; Albertsen et al., 2011; Jiang et al., 2017). Fusions have the potential to facilitate more efficient substrate channeling by maintaining close physical proximity of sequential pathway enzymes, thus limiting competition from host enzymes. In addition to terpenoid systems, fusion of resveratrol biosynthetic enzymes facilitated a 15-fold improvement in titer in engineered *S. cerevisiae* (Zhang et al., 2006). Enzyme fusions have the added potential to improve protein stability or solubility, as demonstrated through fusions of taxadiene synthase with the soluble *E. coli* maltose binding protein, yielding a 25-fold improvement in taxadiene titer (Reider Apel et al., 2017). Fusions with maltose-binding protein also improved production of α-ionone up to 50-fold (Zhang et al., 2018a).

EXPANDING THE PLANT SECONDARY METABOLIC SPACE IN MICROBIAL SYSTEMS

An emerging application of synthetic biology aims to exploit microbial systems for drug discovery. In this manner, plant enzyme promiscuity and combinatorial biochemistry are exploited to synthesize structural scaffolds and functionalizations not found in nature.

A common chemical modification within the pharmaceutical industry is halogenation, in which fluoro or chloro moieties are introduced to drug candidates to modify the physicochemical properties of the compound. Because many natural products derive from amino acids, halogenated analogs can be supplemented to engineered microbes leading to the formation of halogenated products. This strategy was used to feed yeast fluoro- or chloro-Tyr, resulting in the synthesis of halogenated BIA pathway intermediates (Li et al., 2018). Halogenated end products were not detected, suggesting a more stringent substrate specificity of downstream BIA enzymes. Moreover, this method relies on the supplementation of costly amino acid analogs and could be overcome by engineering de novo production of halogenated BIAs.

O-sulfated reticuline has been produced de novo by implementing a human sulphotransferase into *E. coli* engineered for reticuline biosynthesis (Matsumura et al., 2018). Promiscuity of BIA pathway enzymes has also been exploited for the production of innovative scaffolds (such as disubstituted- and spiro-tetrahydroisoquinolines) by harnessing the capacity of norcoclaurine synthase to accept an exceptional range of carbonyl substrates (Lichman et al., 2017). Again, this strategy relies on the supplementation of costly substrates, in this case carbonyl species that are not synthesized by microorganisms. Moving these in vitro assays to robust de novo biosynthetic processes presents a formidable challenge.

OUTSTANDING QUESTIONS

- Can microbial production of plant metabolites compete with traditional methods of chemical synthesis and crop-based manufacturing?
- Pathways for several untapped sub-classes of plant specialized metabolites have been elucidated recently (e.g., modified amphetamines and vinblastine). Can these pathways be reconstituted in a microbial species?
- Other functionalized metabolites of immense pharmaceutical value (e.g., paclitaxel) have proven inaccessible to date. What are the limitations and how can they be overcome to realize the microbial synthesis of these important drugs?
- To what extent will emerging synthetic biology workflows reduce the time and cost of advancing bioprocesses from proof-of-concept to large-scale production?
- Will microbial systems provide a viable avenue for drug discovery through the exploitation of enzyme promiscuity and combinatorial biochemistry?

CONCLUSION

Microbial synthesis provides a fresh avenue for the production of natural plant pharmaceuticals, fragrances, nutrients, and colorants. Although this field is in its infancy and faces many challenges (see Outstanding Questions), the wealth of successes in recent years has solidified microbial biosynthetic production as a viable alternative to natural product extraction and total chemical synthesis. The elucidation of increasingly complex plant secondary pathways continues to drive the reconstruction of longer and more challenging routes in microbial systems. Indeed, the discovery of missing enzymes in several highly sought-after plant-specialized pathways has primed a number of untapped natural products for de novo biosynthesis in a microbial species. Several microbially derived natural products have reached commercialization, and these successes will continue to pave the way for opportunities. The exploitation of microbial systems for drug discovery promises to deliver important new structural scaffolds and chemical functionalizations that have thus far not been observed in nature.

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