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MINI-REVIEW

Downstream reactions and engineering in the microbially reconstituted pathway for Taxol

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Abstract Taxol (a trademarked product of Bristol-Myers Squibb) is a complex isoprenoid natural product which has displayed potent anticancer activity. Originally isolated from the Pacific yew tree (*Taxus brevifolia*), Taxol has been mass-produced through processes reliant on plant-derived biosynthesis. Recently, there have been alternative efforts to reconstitute the biosynthetic process through technically convenient microbial hosts, which offer unmatched growth kinetics and engineering potential. Such an approach is made challenging by the need to successfully introduce the significantly foreign enzymatic steps responsible for eventual biosynthesis. Doing so, however, offers the potential to engineer more efficient and economical production processes and the opportunity to design and produce tailored analog compounds with enhanced properties. This mini review will specifically focus on heterologous biosynthesis as it applies to Taxol with an emphasis on the challenges associated with introducing and reconstituting the downstream reaction steps needed for final bioactivity.

Keywords Taxol · Natural products · Metabolic engineering · Heterologous biosynthesis · *S. cerevisiae* · *E. coli*

Introduction

The term "complex natural products" has traditionally been reserved for those compounds with both extensive biosynthetic pathways and unique final molecular structures (Katz 1997). Examples include the polyketide compound erythromycin A (antibiotic), the nonribosomal peptide compound echinomycin (anticancer), and the isoprenoid compound Taxol (anticancer; Fig. 1). Each is represented by a multi-step biosynthetic scheme which includes coordinated enzymatic activity to produce the final molecular structure. Such complexity poses challenges during initial attempts to elucidate the underlying biosynthetic pathways responsible for compound formation and during early compound molecular characterization efforts. Challenges are also encountered during attempts at pathway engineering and chemical or biological synthesis for the purpose of compound overproduction. Yet, despite this complexity and the resulting challenges, basic and applied research emerged and persists because of the medicinal value associated with such natural products, a property intrinsically associated with the same molecular complexity that complicates characterization and production efforts.

Among the groups of natural products capable of being classified as complex are the isoprenoids. This is a particularly large classification with tens of thousands of examples having been identified including those, like Taxol, which have resulted in blockbuster drug therapies (McGarvey and Croteau 1995; Sacchettini and Poulter 1997). Focusing on the Taxol compound, the final product meets the criteria for complexity as a result of a putative 19 step biosynthetic pathway and a final molecular architecture featuring 11 chiral centers (Croteau et al. 2006) (Fig. 2). The biosynthetic process begins with the intracellular supply of universal isoprenoid substrates isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) followed by polyisoprene formation of a geranylgeranyl diphosphate (GGPP) molecule which is cyclized by the first dedicated enzyme (taxadiene synthase) of the biosynthetic pathway (Wildung

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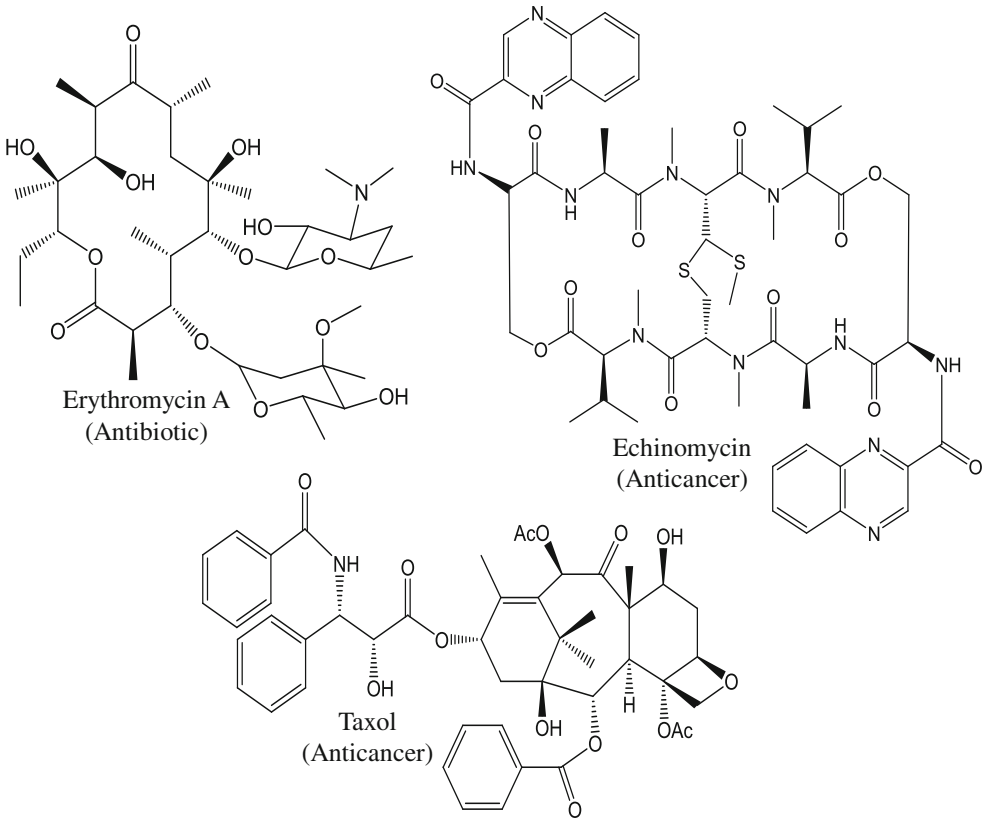

Fig. 1 Representative complex natural products

and Croteau 1996). In the larger context of complex natural product biosynthesis, an early stage intermediate, such as taxadiene, provides a platform for tailoring reactions to produce the final compound. These include reduction, hydroxylation, acetylation, glycosylation, methylation, and other modifications to confer the specific molecular architecture needed for bioactivity (Olano et al. 2010; Walsh et al. 2001). In the case of Taxol, there are reactions dedicated to hydroxylations, oxidation, epoxidation, acetylations, benzoylations, and an amino acid modification and addition. Notably, there is the utilization of several cytochrome P450 hydroxylase enzymes within the Taxol tailoring reactions (Jennewein et al. 2004b; Kaspera and Croteau 2006).

As mentioned, the diverse molecular architectures associated with complex natural products like Taxol are directly responsible for final natural product biological activity. The role of this activity in nature is speculated to be defensive. Foreign organism invasion, cellular damage, and resource limitation have all been linked to natural product formation (Ajikumar et al. 2008; Maplestone et al. 1992). Man-made production efforts are then aimed at harnessing biosynthesis for the purpose of redirecting the medicinal properties of natural products towards human disease, an approach that has been successful and has led to numerous antibiotics, anticancer agents, and other medicines currently available (Newman and Cragg 2007).

However, the therapeutic potential of natural products is often tempered by the non-ideal growth properties of the original hosts responsible for compound formation. Alternative attempts to chemically synthesize the compound must contend with the significant challenges in mimicking natural chemistry, leaving cell-based biosynthesis as the only economical route to production. The slow-growing or fastidious nature of most original cellular producers acts as a final degree of complexity associated with medicinally relevant natural products. Many natural product producers are soil- or marine-dwelling microorganisms. In the case of Taxol and other isoprenoids, plants are a significant source of compounds. Each of these native producers is fastidious in nature, slow-growing, difficult to implement into scaleable production processes, or a combination of these traits. Furthermore, many other natural product producers (>99 %) are considered unculturable (Amann et al. 1995), supporting the viewpoint that the potential of encoded natural product pathways is greatly complicated by the native host producers.

Interestingly, the most technically convenient microbial hosts like *Escherichia coli* and *Saccharomyces cerevisiae*, which have been used widely across the biotech industry, have little propensity for natural product biosynthesis. In the context of heterologous biosynthesis, this lack of production has both advantages and disadvantages (Zhang et al. 2011a). First, while there is little question that successful

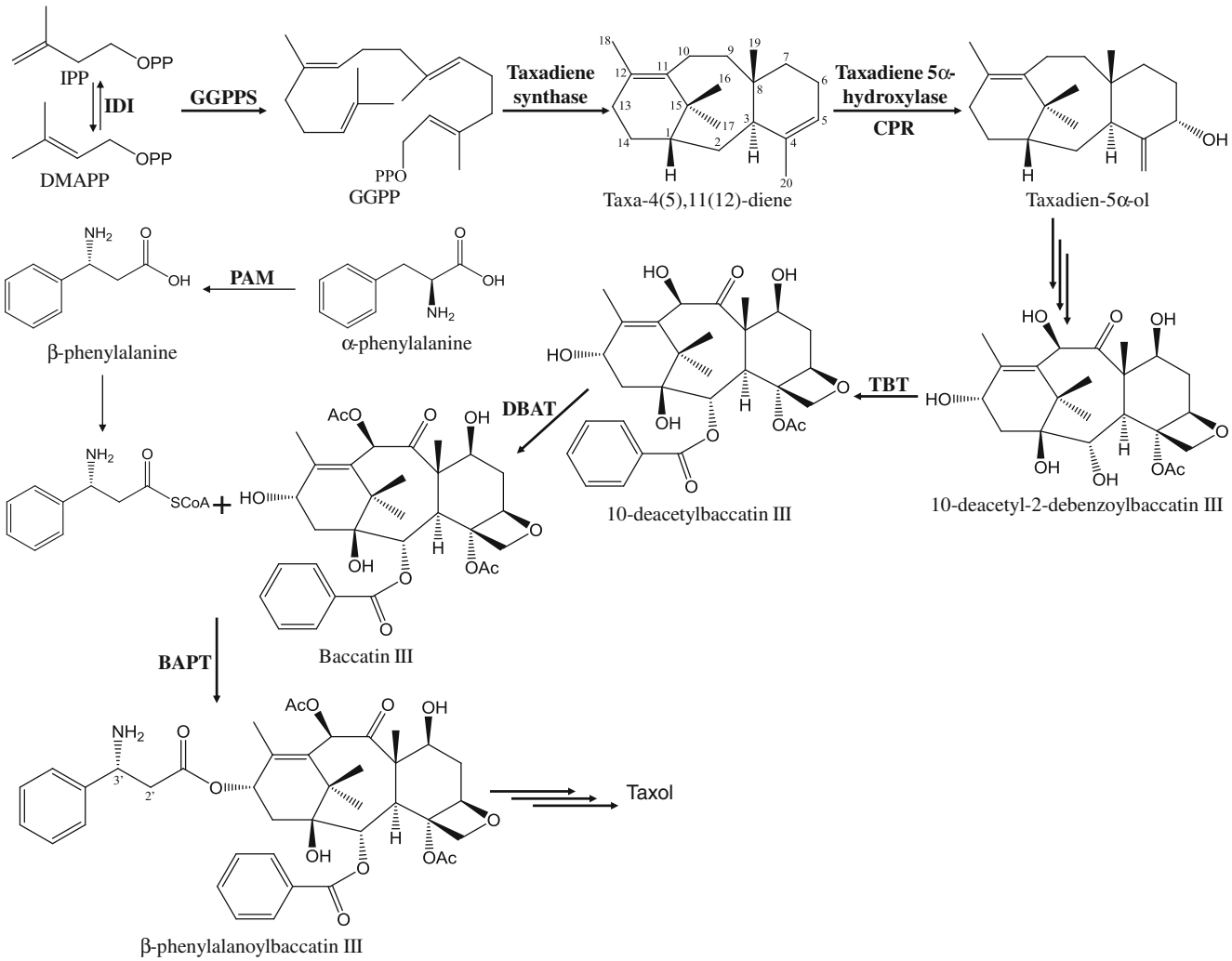

Fig. 2 The downstream Taxol biosynthetic pathway. Those enzymes shown have demonstrated activity either biochemically or biosynthetically towards the indicated substrate and include geranylgeranyl diphosphate synthase (GGPPS; Hefner et al. 1998), taxadiene synthase (Wildung and Croteau 1996; Koepp et al. 1995; Huang et al. 2001), taxadiene 5α-hydroxylase (Jennewein et al. 2004a), taxane 2α-*O*-benzoyltransferase (*TBT*; Walker and Croteau 2000b), 10-deacetylbaccatin III-10-*O*-acetyl transferase (*DBAT*; Walker and Croteau 2000a), baccatin III:3-amino-3-phenylpropanoyltransferase (*BAPT*; Walker et al. 2002a), and phenylalanine aminomutase (*PAM*; Walker et al. 2004). Other enzymes (taxadien-5α-ol-*O*-acetyl transferase, taxoid 10β-hydroxylase, taxoid 2α-hydroxylase, taxoid 9α-hydroxylase, taxane 13α-hydroxylase, taxoid 7β-hydroxylase, and 3′-*N*-debenzoyl-2′-deoxytaxol *N*-benzyoyltransferase) have been identified and/or characterized but not conclusively assigned a location in the overall pathway order (Walker et al. 2000; Schoendorf et al. 2001; Chau and Croteau 2004; Croteau et al. 2006; Jennewein et al. 2001; Chau et al. 2004; Walker et al. 2002b). Still other enzymes (taxoid 1β-hydroxylase, taxoid 9-keto-oxidase, β-phenylalanoyl-CoA ligase, taxoid 2′-hydroxylase, and those involved with oxetane ring formation) have not been identified. *IDI* isopentenyl diphosphate isomerase, *CPR* cytochrome P450 reductase

reconstitution of a complex, therapeutically relevant natural product pathway in a convenient microbial heterologous host would aid basic and applied studies, the lack of prerequisite metabolic support increases the challenge of successful reconstitution. For example, a separate metabolic engineering strategy may be required to introduce or enhance substrates required by downstream biosynthetic enzymes. Alternatively, the lack of native natural product pathways may (1) simplify efforts to regulate the introduced pathway; (2) require less metabolic engineering to eliminate "cross talk" with existing natural product pathways; and (3) result in a cleaner purification process without contaminating native products.

Over the last 10 years, several studies have focused on the production of complex plant-derived isoprenoids through *E. coli* and *S. cerevisiae*. This review will place particular emphasis on the Taxol biosynthetic pathway and the tailoring reactions needed to generate final bioactivity. Considering that the current state-of-the-art is the production of early stage Taxol intermediates through heterologous hosts, this discussion will summarize success to date and the steps needed to realize and capitalize upon full pathway reconstitution.

Heterologous biosynthetic logic: upstream and downstream components

Being confined to a cellular environment, heterologous production attempts begin with the basic substrates, cofactors, and other metabolites needed to support biosynthesis. Isoprenoid biosynthesis substrates can be derived from one of two pathways, and both have been used in previous heterologous settings. The mevalonate (MVA) pathway, native to *S. cerevisiae*, begins with acetyl-CoA (Bloch 1992; Miziorko 2011); whereas, the alternative methylerythritol phosphate (MEP) pathway, native to *E. coli*, begins with a molecule each of pyruvate and glyceraldehyde 3-phosphate (Grawert et al. 2011; Rohmer 1999). These substrates can be linked to primary metabolic pathways that must be re-routed when triggered (either natively or heterologously) for isoprenoid biosynthesis. The formation of the IPP and DMAPP by the MVA or MEP pathways is considered the upstream portion of overall biosynthesis. In the context of heterologous metabolic engineering, in which the objective is to establish production of a non-native isoprenoid compound, the substrate provision pathways must minimally be able to support even the smallest level of eventual final product formation. Whereas, in pathway metabolic engineering, the goal is to maximize specific productivities and the upstream pathway must then be optimized to support final compound overproduction.

The downstream portion of isoprenoid biosynthesis are those reactions dedicated to polyisoprene formation, template compound cyclization, and template compound tailoring. Unlike the upstream pathway, there is generally no direct link to primary metabolism and the introduced metabolism is foreign. As such, there are a series of precautions to be taken to aid the process of functional gene expression. These challenges derive primarily because of the cell-specific differences between native and heterologous hosts. As an example, the genetic, physiological, and structural differences between a plant cell and an *E. coli* cell impact everything from gene expression templates and parameters to physical enzyme location post-expression.

With regards to Taxol, both *S. cerevisiae* and *E. coli* have been used to generate early stage intermediates in the downstream pathway (Ajikumar et al. 2010; Dejong et al. 2006; Engels et al. 2008; Huang et al. 2001). Both hosts are strong candidates for heterologous biosynthesis as a function of their rapid growth rates (compared to plant cells) and range of molecular biology tools to facilitate and optimize metabolic engineering efforts. Given the fact that the entire pathway has yet to be incorporated into either host, it is unknown which will serve the purpose of complete Taxol heterologous biosynthesis. The choice may then be decided by individual researcher preference or biased by host- or pathway-specific features. Over the last 10 years, the emergence of facilitating technical developments for hosts like *E. coli* and *S. cerevisiae* have continually fueled and allowed increasing attempts at heterologous natural product biosynthesis with great success. For example, gene synthesis and chromosomal engineering methodology helped expedite the heterologous transfer and reconstitution process for artemisinin (an antimalarial compound) and Taxol intermediate production (Ajikumar et al. 2010; Martin et al. 2003; Ro et al. 2006). The downstream pathways for complex isoprenoid compounds typically feature several steps catalyzed by plant cytochrome P450 enzymes and a partner reductase (Croteau et al. 2006; Jennewein et al. 2005; Kaspera and Croteau 2006). Besides the usual challenges in establishing functional enzymatic activity in a heterologous host, these enzymes are membrane bound and may benefit from a heterologous cellular structure similar to that found in plants. From this perspective, *S. cerevisiae* would be at an advantage relative to *E. coli*. However, similar to the advances that have aided biosynthetic efforts to this stage, should new technology allow for efficient and functional expression of a series of such complicated enzymes within either *E. coli* or *S. cerevisiae*, both hosts would then be viable options for heterologous biosynthesis.

Challenges and options associated with the heterologous implementation of the Taxol downstream biosynthetic pathway

The challenges facing heterologous Taxol formation through microbial hosts mirror those for other complex natural products. Namely, genetic material must be available for transfer, successful gene expression and enzymatic activity must be accomplished in the new host, and sensitive analytical capabilities must be available to characterize and quantify compound formation. The process is aided by a working knowledge of the biochemical steps in product formation and experimental options to address the many issues that can arise during heterologous production attempts. In this regard, current work took advantage of previously established results in Taxol pathway analysis and emerging technology available with microbial hosts to assist reconstitution efforts.

The first requirement in establishing heterologous biosynthesis is to have access to the native biosynthetic genes responsible for product formation. Given the medicinal value of natural products like Taxol, significant investment has been made to elucidate the responsible biosynthetic pathway. On this front, Rodney Croteau's group at Washington State University made significant advances in identifying genes of the Taxol pathway and testing expression in heterologous hosts.

Early efforts to identify the exact biosynthetic steps towards Taxol's production were published in the mid to late 1990s (Hefner et al. 1998, 1996; Hezari et al. 1995; Koepp et al. 1995; Walker et al. 1999; Wildung and Croteau 1996). This work identified the geranylgeranyl diphosphate synthase, taxadiene synthase, cytochrome P450 hydroxylase, and acetyltransferase needed to make the hypothesized first three dedicated intermediates of the Taxol pathway. Four of these six initial isolation studies were conducted with native *Taxus* plant sources providing the enzymes of interest. A concerted attempt was then made to generate cDNA libraries from *Taxus* cell cultures and to begin identifying and confirming subsequent pathway genes (Chau and Croteau 2004; Chau et al. 2004; Huang et al. 2001; Jennewein et al. 2001, 2004b; Long and Croteau 2005; Schoendorf et al. 2001; Walker and Croteau 2000a, b; Walker et al. 2002a, b, 2000, 2004).

These results were commendable from several viewpoints. First, the work helped establish a hypothetical biosynthetic path to the final Taxol molecule (Fig. 2), while simultaneously providing the genetic blueprint to consider reconstructing the downstream pathway in heterologous hosts. Both *E. coli* and *S. cerevisiae* were used throughout studies to overproduce and characterize pathway enzymes. On occasion, insect (*Spodoptera frugiperda* 9) hosts were used as alternatives to achieve higher expression levels or when problems emerged with the more convenient heterologous hosts (Chau et al. 2004; Jennewein et al. 2004a, 2005, 2001). In each of these examples, a pathway P450 or P450 reductase was also tested for expression in *S. cerevisiae*. Generally, Croteau's group favored *S. cerevisiae* for P450 heterologous expression (Jennewein et al. 2003, 2004a; Schoendorf et al. 2001; Chau et al. 2004), most likely owing to the similarities to the native plant host. *E. coli* was prominently featured as an expression host with other pathway genes (Huang et al. 1998; Walker and Croteau 2000a, b; Walker et al. 2002a, b, 2000, 2004). Each of these heterologous expression attempts set the stage for heterologous biosynthetic production.

Not surprisingly, Croteau's group took the lead in attempting to produce intermediates from the Taxol pathway through technically convenient heterologous hosts. The first attempts used *E. coli* to express the taxadiene synthase gene for the purpose of producing the first Taxol pathway intermediate (Huang et al. 2001). Prior to this success, *E. coli* taxadiene synthase expression and activity was systematically evaluated across gene expression parameters and hosts (Huang et al. 1998; Wildung and Croteau 1996; Williams et al. 2000). The T7 promoter expression system was featured in these attempts; however, variations were made according to the strains tested for expression (including K vs. B lineages). Expression was also optimized to avoid inclusion body formation and/or inactivity by the use of fusion proteins and the removal of an N-terminal signaling peptide which was specific for subcellular membranes in plant cells. These approaches, coupled with induced expression at reduced temperatures (20–25°C), resulted in much improved enzyme levels and activity. However, before heterologous taxadiene production was achieved, separate genes encoding for MEP pathway steps or an *Erwinia herbicola* geranylgeranyl diphosphate synthase were required to allow final taxadiene titers of 1.3 mg/L.

An ambitious attempt at *S. cerevisiae* heterologous production was made through the coordinated expression of the first five hypothesized steps in Taxol formation (from IPP and DMAPP). The geranylgeranyl diphosphate synthase and taxadiene synthase were again truncated to remove plant-specific signal sequences; whereas, constructs for the expected next three steps (taxadiene 5α-hydroxylase, taxadien-5α-ol-*O*-acetyl transferase, and taxoid 10β-hydroxylase responsible for taxadien-5α-ol, taxadien-5α-yl-acetate, and taxadien-5α-acetoxy-10β-ol, respectively) were used based upon successful previous expression attempts in either *E. coli* or *S. cerevisiae* (Jennewein et al. 2004a, 2001; Schoendorf et al. 2001). Taxadiene titers were reported at 1.0 mg/L though only trace amounts of the second intermediate (taxadien-5α-ol) were observed (Dejong et al. 2006); taxadien-5α-yl-acetate and taxadien-5α-acetoxy-10β-ol were not conclusively identified, emphasizing the uncertainty with the overall pathway past taxadien-5α-ol formation (Fig. 2). A later effort by Stefan Jennewein's group to optimize the heterologous production of taxadiene from *S. cerevisiae* was notable for the application of metabolic engineering to improve biosynthetic substrate availability, the use of a codon-optimized taxadiene synthase gene, and final taxadiene levels of 8.7 mg/L (Engels et al. 2008).

However, in either case, whether for biochemical characterization or heterologous production, ensuring successful gene expression in foreign hosts is not trivial. The body of work presented above served as a valuable basis for recent attempts at heterologous Taxol (or other isoprenoid) production attempts. The most recent efforts derive both from the focused work on Taxol biosynthesis and the more general advances in metabolic engineering and gene expression that have occurred over the last 10 years. A combination of metabolic engineering and optimized gene expression led to improved (~300 mg/L) production of taxadiene from *E. coli* (Ajikumar et al. 2010). Here, metabolic engineering was used to balance the flow of carbon between upstream and downstream pathways as a way to optimize final titers. However, when establishing the downstream pathway within a foreign host, great care must be taken to correctly design the newly introduced gene(s) for successful expression. Croteau's group recognized this by removing plastidial regions from the 5′ regions of genes to be expressed in *E.*

Table 1 Genetic, protein, and process modifications to facilitate high titer production of taxadiene and taxadien-5α-ol from *E. coli*

	Gene synthesis	N-terminal peptide removal	N-terminal peptide addition	Linker region to produce fusion protein	Reduced temperature culture conditions	Bioreactor production
TXS and GGPPS	✓	✓			✓	✓
P450 and CPR	✓	✓	✓ (P450 only)	✓	✓	✓

TXS taxadiene synthase, GGPPS geranylgeranyl diphosphate synthase, P450 cytochrome P450, CPR cytochrome P450 reductase

coli. In addition to this step, the approach by Ajikumar et al. featured gene synthesis, gene dosage levels (chromosomal and varying copy number plasmid expression), promoter variation (T7, T5, and trc), and process temperature to influence and optimize final enzyme function in the context of heterologous biosynthesis. The combination of metabolic engineering and gene expression technology, together with the prior knowledge provided by the efforts of the Croteau group, allowed high level taxadiene formation from *E. coli* (Table 1).

The engineering approaches mentioned above were first used to maximize *E. coli* taxadiene formation. However, subsequent P450 steps were made more complicated for several reasons. First, these enzymes are membrane associated with the endoplasmic reticulum of native hosts (Bernhardt 2006). Approximating this membrane localization in *E. coli* will clearly be challenging. However, taking the lead from numerous groups that have focused on *E. coli* gene expression of mammalian and plant P450 enzymes (Barnes 1996; Barnes et al. 1991; Chang et al. 2007; Jenkins et al. 1998; Leonard et al. 2006; Pritchard et al. 2006; Yun et al. 2006), functional expression of the first Taxol pathway P450 was accomplished (Ajikumar et al. 2010). Doing so required truncation of the native transmembrane region (with three different truncation lengths tested) and further modification of the N-terminal portion of the enzyme to include an eight amino acid membrane anchor peptide sequence (MALLLAVF) which has proven functional in *E. coli* (Barnes et al. 1991). Furthermore, as in the case of the taxadiene synthase, the P450 gene was synthesized to eliminate any codon bias (this same step was also taken for the accompanying P450 reductase), and reduced temperature induction conditions and other process parameters were modified so as to maximize eventual production (Table 1).

An additional complication of the Taxol P450 enzymes is the need for a dedicated reductase (CPR) partner. This can be mitigated by the native capabilities to complement reductase activity when using *S. cerevisiae*, but the same level of convenience may not be provided with *E. coli* (Barnes et al. 1991; Jenkins and Waterman 1998; Jennewein et al. 2005; Oeda et al. 1985). Again thanks to work by Rodney Croteau's group, a reductase has been isolated from *Taxus cuspidata* (Jennewein et al. 2005). The reductase was also modified by N-terminal transmembrane region removal prior to covalently coupling the reductase to the P450 through a linker peptide (Ajikumar et al. 2010), as a means to co-localize the enzyme pair and to mimic highly active fusion P450-reductase examples found in nature (Fisher et al. 1992; Fisher et al. 1996; Leonard et al. 2006; Munro et al. 2002; Narhi and Fulco 1982). A series of codon-optimized, truncated fusion P450-reductase pairs were then tested for coupling to the previously optimized metabolism for *E. coli* taxadiene formation with titers of taxadien-5α-ol reaching 58 mg/L. Productions efforts also revealed, however, the formation of an unwanted hydroxylated isomer, which suggests inefficiencies in expression design and/or protein activity. This unwanted side product presents a current challenge in optimizing production through *E. coli*. However, given successful previous efforts to apply protein engineering to P450 enzymes (Grogan 2011; Urlacher and Girhard 2011), there is reason to believe similar strategies could be applied for the purpose of improving the specific productivity of taxadien-5α-ol using the *E. coli* system.

Table 2 Representative complex natural products heterologously produced through *E. coli*

Compound	Total biosynthetic steps[a]	Heterologous product (biosynthetic number of steps)	Number of P450 enzymes introduced	Final titer (mg/L)	References
Erythromycin A[b] (polyketide)	20	Erythromycin A (20)	2	4	(Zhang et al. 2011b, 2010)
Echinomycin[b] (nonribosomal peptide)	14	Echinomycin (14)	1	0.3	(Watanabe et al. 2006)
Taxol (isoprenoid)	19	Taxadien-5α-ol (2)	1	58	(Ajikumar et al. 2010)

[a] Not including those steps required to provide the starting substrates for compound biosynthesis. Number for Taxol begins from geranylgeranyl diphosphate and is considered putative as not all steps have been conclusively identified

[b] Both systems include modular enzymes responsible for catalyzing multiple reaction steps. For simplification, these enzymes have been counted as one step such that the total number of steps is equal to the number of heterologous enzymes required

In summary, the last 15 years have seen increasing efforts to heterologously produce complex isoprenoid compounds using hosts such as *E. coli*. In the case of the Taxol system, the combination of an emerging biosynthetic pathway and improvements in the expression technology available to transfer this pathway into alternative hosts has led to successful production of the first two dedicated pathway intermediates. The question now turns to complete pathway implementation and optimization for sufficient heterologous production and which cellular platform will eventually better serve this purpose.

Complete biosynthesis: a combination of heterologous and pathway metabolic engineering

The eventual goal of heterologous biosynthesis is to produce the original natural product and designed analogs at titers sufficient to allow mass distribution. This can only be done with successful metabolic engineering, both to establish initial production and then to maximize the flow of carbon through upstream and downstream biosynthetic components. Initial reconstitution has been accomplished for complex polyketide and nonribosomal peptide natural products, with both examples requiring multiple tailoring reactions to complete biosynthesis (Table 2). In these cases, pathway metabolic engineering has not yet allowed overproduction. In other words, though each final natural product has been impressively generated through a surrogate host, current titers are too low to present a viable production route.

Interestingly, pathway metabolic engineering has been used successfully to generate substantial titers of heterologous isoprenoid compounds (Ajikumar et al. 2010; Chang et al. 2007; Ro et al. 2006). However, these compounds have only been intermediates in the overall downstream pathways. In the case of the Taxol intermediate taxadiene, grams per liter titers have been accomplished through careful balancing of the upstream and downstream biosynthetic pathways coupled to small-scale bioreactor systems (Ajikumar et al. 2010). The artemisinin intermediate amorphadiene has also been produced at grams per liter levels through the application of metabolic and process engineering (Martin et al. 2003; Tsuruta et al. 2009). Production of the remaining downstream intermediates for both compounds requires the introduction of P450 and other tailoring enzymes and has not reached the same concentration levels associated with taxadiene and amorphadiene (although it should be noted that the artemisinin intermediate artemisinic acid (Ro et al. 2006) could be produced and linked to a semi-synthetic strategy to generate the full compound).

This scenario points to the challenges in establishing complete and sufficient biosynthesis, especially when unique biosynthetic steps are introduced to a significantly different host system. Furthermore, in the case of Taxol, several steps in the overall pathway have not been conclusively identified or confirmed. As such, research must continue to be directed at strategies to discover and characterize missing pathway enzymes. However, if the heterologous route is ever to become viable, these challenges must be overcome. In addition, high titer production must also be reproducible, non-toxic to the new host (allowing high-cell-density bioprocesses), and adjustable for the production of rationally designed analogs. In the case of Taxol, heterologous biosynthesis would allow several opportunities. First, an *E. coli* or *S. cerevisiae* production platform (either for the final compound or an intermediate capable of being converted to the final compound via semi-synthesis) would offer a potentially faster and more economical route to the current Taxol product. Second, reconstitution offers the opportunity to directly alter biosynthesis for the purpose of producing new analogs with improved potency and a broader therapeutic spectrum (Long et al. 1998; Woo et al. 1994; Young et al. 1992; Zhang et al. 2005) or for the purpose of improving post-administration in vivo bioavailability (a problem that has plagued the hydrophobic Taxol molecule (Hennenfent and Govindan 2006)).

This is the vision of heterologous biosynthesis. It will be interesting to see where the field stands in another 10 years. Now, with the advantage of hindsight, it is clear the approach can be used for the production of complex natural compounds, most notably, polyketides and nonribosomal peptides. Over the next decade, the continued application of metabolic engineering concepts will decide the range of compounds to be completely biosynthesized and the production titers that would support eventual process viability.

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