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Current and Emerging Options for Taxol Production

Yi Li, Guojian Zhang and Blaine A. Pfeifer

Abstract Paclitaxel (trademark "Taxol") is a plant-derived isoprenoid natural product that exhibits potent anticancer activity. Taxol was originally isolated from the Pacific yew tree in 1967 and triggered an intense scientific and engineering venture to provide the compound reliably to cancer patients. The choices available for production include synthetic and biosynthetic routes (and combinations thereof). This chapter focuses on the currently utilized and emerging biosynthetic options for Taxol production. A particular emphasis is placed on the biosynthetic production hosts including macroscopic and unicellular plant species and more recent attempts to elucidate, transfer, and reconstitute the Taxol pathway within technically advanced microbial hosts. In so doing, we provide the reader with relevant background related to Taxol and more general information related to producing valuable, but structurally complex, natural products through biosynthetic strategies.

Keywords Paclitaxel · *Taxus* · Metabolic engineering · *E. coli* · Yeast · Fungi

Abbreviations

BMS	Bristol-Myers Squibb
DOE	Design of experiments
DMAPP	dimethylallyl diphosphate
DXS	1-deoxy-D-xylulose 5-phosphate synthase
FDA	Food and Drug Administration
FPP	farnesyl diphosphate
FPPS	farnesyl diphosphate synthase
GGPPS	geranylgeranyl diphosphate synthase
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
IPP	isopentenyl diphosphate

Y. Li · G. Zhang · B.A. Pfeifer (✉)
Department of Chemical and Biological Engineering,
The State University of New York at Buffalo, Buffalo,
NY 14260, USA
e-mail: blainepf@buffalo.edu

ispD	4-diphosphocytidyl-2-*C*-methyl-D-erythritol synthase
ispF	2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase
IDI	isopentenyl diphosphate isomerase
IND	Investigational new drug
MEP	2*C*-methyl-D-erythritol-4-phosphate
MVA	Mevalonate
NCI	National Cancer Institute

Contents

1 Introduction

The isolation and elucidation of the Taxol compound began two separate journeys based upon the compound's medicinal value and impact. First, there was a clinical goal of formulating and administering the compound for anticancer application. In this capacity, the compound has seen tremendous success [1, 2]. The international market for Taxol production reached $1.6 billion in 2005 [3]. Such financial outcomes established Taxol as the worldwide best-selling anticancer drug, regardless of direct source or semisynthetic production routes [4]. Furthermore, as a cytotoxic chemotherapy drug with high response rates, Taxol is widely used for not only various types of solid tumors associated with breast, ovarian, lung, and bladder cancer but also in treatment therapies for Kaposi's sarcoma [5–8]. In parallel, a second effort also began towards process development for improved accessibility to the compound.

Originally, Taxol was isolated from the Pacific yew tree *Taxus brevifolia* through an ambitious program initiated by the NCI in 1958 to screen 35,000 plants for anticancer activity [9]. Once enough Taxol had been harvested to confirm medicinal potency fully and allow full chemical structural characterization [10], efforts began towards scalable production of the Taxol molecule. There are several options towards this end including a purely synthetic route [11]. However, the structural complexity of Taxol, like many other complex natural products, places limits on a synthetic production option that must also be affordable and scalable.

In other words, synthetic routes have been established, but the multiple steps needed and the subsequent loss in yield at each step complicate economic production.

Alternatively, the native biological host could be considered a vessel for production purposes. From this perspective, additional consideration would have to be given to the biological constraints of the native producer. Insurmountable or very challenging obstacles associated with native host production has spurred further and more recent production alternatives that include an approach termed heterologous biosynthesis [12, 13]. In this capacity, the biosynthetic steps required for Taxol formation are transferred from the native and typically more fastidious host and implemented in more technically friendly microbial hosts possessing both innate biological properties and engineering tools to facilitate pathway reconstitution and compound overproduction. However, the process of establishing heterologous biosynthesis also presents a set of technical challenges that must be overcome. As such, the following chapter is dedicated to summarizing Taxol production with an emphasis on options that are currently used and those that may one day supplant them.

2 Taxol Origins and Production Development

2.1 Natural Role and Therapeutic Utilization

Although clearly established as a cancer chemotherapeutic, the native role of Taxol is much less clear. The mechanism of anticancer activity is tubule stabilization and, hence, cell-cycle arrest [14, 15]. Hypothetically, this same activity could serve in a defensive role during events that threaten plant health. For example, Taxol showed particular activity towards invasive oomycetes across various taxonomic groups [16]. In studies designed to assess the impact of altering compound side-chain functionality, activity was also demonstrated against *Phytophthora capsici* and *Aphanomyces cochlioides*, two plant pathogens [17].

These same properties, however, endowed the compound as a strong neoplastic agent. *Taxus brevifolia* crude plant extract was found to be cytotoxic in cellular assays conducted in the early to mid-1960s [4]. Monroe Wall then isolated and named the active Taxol component from fresh *Taxus* samples in 1967 [9]. In early 1979, Susan Horwitz demonstrated that Taxol had a previously unknown mechanism of action involving the stabilization of microtubules [18]. Upon collection of 20,000 lbs of *Taxus* bark for compound isolation, animal toxicology studies were complete by June 1982, and in November, the NCI submitted an IND application to begin clinical trials [19]. Early clinical trials began in April 1984, and in March 1988 the FDA released the final results of Phase II trials against the most aggressive forms of ovarian cancer with a response rate that averaged 30 % [20]. The FDA first approved Taxol for the treatment for ovarian cancer in 1992 [21]. FDA approval for breast cancer and Kaposi's sarcoma was accomplished in 1994 and 1997, respectively [22, 23].

2.2 Production Based upon Plant Organisms

The identification of Taxol as a potent anticancer agent provided the impetus for early production efforts. Assays and tests designed to characterize and assess the Taxol compound further could rely on the simplest isolation routes that involved direct extraction of the compound from the bark of the yew tree. Once these studies confirmed the potential of the molecule, more refined approaches were required for the processes needed to enable widespread distribution.

It readily became evident that bark extraction could not economically support large-scale production attempts. Furthermore, the environmental impact for such a strategy was unacceptable. To treat one patient effectively, the bark of two to three yew trees was required with each fully grown tree only able to provide 0.5 g of Taxol [24]. Considering that each extraction process was destructive to the tree source and mature yews required 200 years to develop fully, this form of direct plant isolation was clearly inefficient and nonsustainable.

However, a compromise was reached regarding direct yew tree Taxol isolation. Namely, a key precursor from the Taxol pathway, Baccatin III (Fig. 1), was readily extracted from yew tree needles in a manner that was nondamaging to the overall health of the tree [25, 26]. Although this intermediate did not provide the same therapeutic value of Taxol, the late-stage precursor could be readily converted to the full Taxol molecule through separate synthetic chemistry transformations [27, 28].

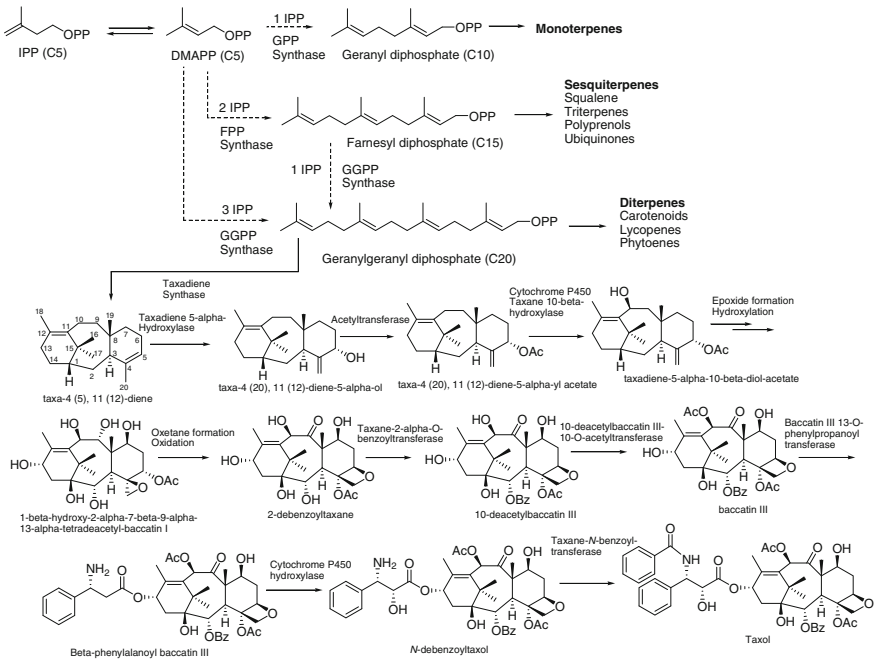

Fig. 1 Biosynthetic path to Taxol

Hence, a semisynthetic route to Taxol was achieved. Indeed, the strategy proved effective in large-scale Taxol production. As such, BMS became the industrial partner involved with mass production and led an effort towards commercialization by signing a cooperative research and development agreement with Robert Holton, who developed the semisynthetic route to Taxol, at Florida State University [28].

Over the course of the research to establish the semisynthetic route to Taxol, there remained interests in alternative production options. In particular, although the semisynthetic strategy proved viable, there was still the need to rely on macroscopic tree components and direct extraction from such sources. One option more compliant with developing bioreactor strategies for biological products was plant cell culture. In this context, early attempts at cell suspensions were initiated by conditioning macroscopic fragments of *Taxus calli* for liquid-phase growth, which led to culture-based production and optimization strategies [29, 30]. The first reported *T. baccata* callus induction and culture was reported in 1973 [31].

Cell culture-based process development strategies allowed the transition to a unit operation-based form of production and the associated application of process engineering strategies as outlined in Table 1. Cell culture variability and low product yields are two limitations for plant cell culture technology [32]. In addition, secondary metabolite accumulation has been correlated with negative cellular effects resulting in culture growth inhibition and overall production instability. At the cellular population level, such phenotypic pressure results in groups of aggregates and heterogeneity, contributing to production variability [33, 34]. To help address these issues and effectively increase paclitaxel production, strategies such as precursor and substrate feeding, media composition modification [35], two-phase partitioned organic solvent extraction, elicitor addition (including agents such as chitosan, silver ion, methyl jasmonate), and cellular dispersion (ultrasound) have been attempted. Other strategies utilizing solid adsorbants (e.g., polymeric resins of the nonionic Amberlite XAD series [36]) as well as self-immobilized aggregates of *Taxus cuspidata* [37] have also been tested as ways to improve overall production.

For larger-scale production of paclitaxel, optimized bioreactor designs and configurations are favored. Elicitation and immobilization strategies had been applied to bioreactors operated in stirred, airlift, pneumatic, and wave formats [46]. Currently, all paclitaxel production for BMS uses plant-cell fermentation technology developed by the Ithaca, New York biotechnology company Phyton Biotech, Inc. and carried out at their production plant in Germany [42]. Phyton Biotech currently employs up to 75,000 L bioreactors for commercial production of paclitaxel from cell culture [47].

2.3 Taxol Pathway Elucidation

Plant-derived Taxol biosynthesis remains a viable option for production purposes. However, there was both a basic interest and applied rationale for identifying the dedicated pathway required for biosynthesis. Scientifically, the enzymology behind

Table 1 Plant-cell culture-based production strategies

Cellular source	Engineering strategy	Vessel format	Compound titer (mg/L)	Reference
Taxus baccata	Immobilization within Ca^{2+}—alginate beads	Stirred bioreactor	43.43 at day 16	[38]
		Wave bioreactor	20.79 at day 8	
	Methyl jasmonate-induced and silver thiosulfate (antiethylene) addition	Large-scale two-stage process	295	[39]
	Jasmonate-induced	Shake flask	48.3 at day 14	[40]
Taxus chinensis and endophytic fungi	Reparable separation membrane	Cobioreactor	25.63 at day 15	[41]
Taxus chinensis	Combination of enhancement agents and medium exchange	Fed batch	900	[42]
	Chitosan, methyl jasmonate-induced, and Ag^{+} addition	Shake flask	25	[43]
	Mechanical stimulus, ultrasound, methyl jasmonate-induced, in situ solvent extraction	Shake flask	33–35	[44]
Taxus cuspidata	Self-immobilization aggregate	Shake flask	4.9 at day 40	[37]
	Medium composition modification	Shake flask	431 at day 55	[45]

biosynthesis would contribute to a growing number of understood complex natural product pathways and the mechanisms behind individual intermediate transformations. Such insight then lends itself to applied efforts to modify or utilize resulting information for unique production purposes. For example, an understanding of the dedicated biosynthetic steps would allow intervention for the purpose of altering pathway steps and eventual final products, which may, in turn, possess new biological activity.

Alternatively, complete knowledge of the Taxol biosynthetic pathway would allow the potential for heterologous biosynthesis through an alternative production host. Notwithstanding the production options offered by plant systems, the constraints of original production routes suggest an opportunity to harness production through technically amenable microbial hosts. This basic theme is the genesis for most interests and efforts in heterologous biosynthesis.

Towards this end, Rodney Croteau's group at Washington State University took the lead in elucidating the steps required for Taxol biosynthesis [48–53]. The important outcome of this research was an outline of a hypothetical path to Taxol biosynthesis that could be utilized in the context of basis studies to understand enzymatic conversion steps and applied attempts to heterologously reconstitute the biosynthetic pathway. However, towards the latter goal, a significant challenge beyond the obvious technical steps in designing reconstitution is the fact that several steps remain unknown in the biosynthetic path to Taxol (Fig. 1). In particular, genes encoding taxoid 1β-hydroxylase, taxoid 9-keto-oxidase, β-phenylalanoyl-CoA ligase, taxoid 2′-hydroxylase, epoxidation reactions at C4 and C5, and those involved in oxetane formation are still to be identified. Thus, more research is required to identify these steps before reconstitution efforts can be planned with complete confidence.

2.4 Microbial Production

With the partial elucidation of the Taxol biosynthetic pathway came early attempts towards heterologous biosynthesis. As a basis for such efforts, the new hosts must provide the prerequisite universal isoprenoid substrates needed to support downstream compound biosynthesis. The two routes available for this purpose include the MVA and nonmevalonate (or MEP) pathways (Fig. 2) [54]. The MVA pathway is generally found in eukaryotic organisms; whereas, the MEP pathway is commonly associated with prokaryotic metabolism [55, 56]. Before biosynthesis can commence, a substrate support pathway must be available and, in most cases, engineered to support isoprenoid overproduction.

However, the engineering dedicated to pathway support must be completed with care. Otherwise, the buildup of intermediates will lead to global cellular toxicity. For example, prenyl pyrophosphate intermediates such as IPP, DMAPP, and FPP involved in the biosynthetic pathway towards Taxol have demonstrated intracellular toxicity within microbial systems [57]. In addition, HMG-CoA from the alternative MVA pathway has resulted in negative cellular effects upon fatty acid biosynthesis [58].

Upon completing the cellular engineering to support broad isoprenoid biosynthesis, the dedicated enzymes required for product formation will subsequently be introduced. From this perspective, the downstream steps to isoprenoid formation can be subdivided into those reactions required for terpene synthesis (the hydrocarbon portion of the final isoprenoid compound) and those steps required for terpene tailoring. The first series of reactions can include both isoprene elongation steps, which dictate the classification of the final isoprenoid compound (Fig. 1), and a terpene synthase step that cyclizes the elongated terpene chain into a configurational core structure [59]. The isoprenoid tailoring reactions then proceed to decorate the resulting terpene compound with functional units that confer key bioactivity. These reactions, particularly featured in Taxol formation, include

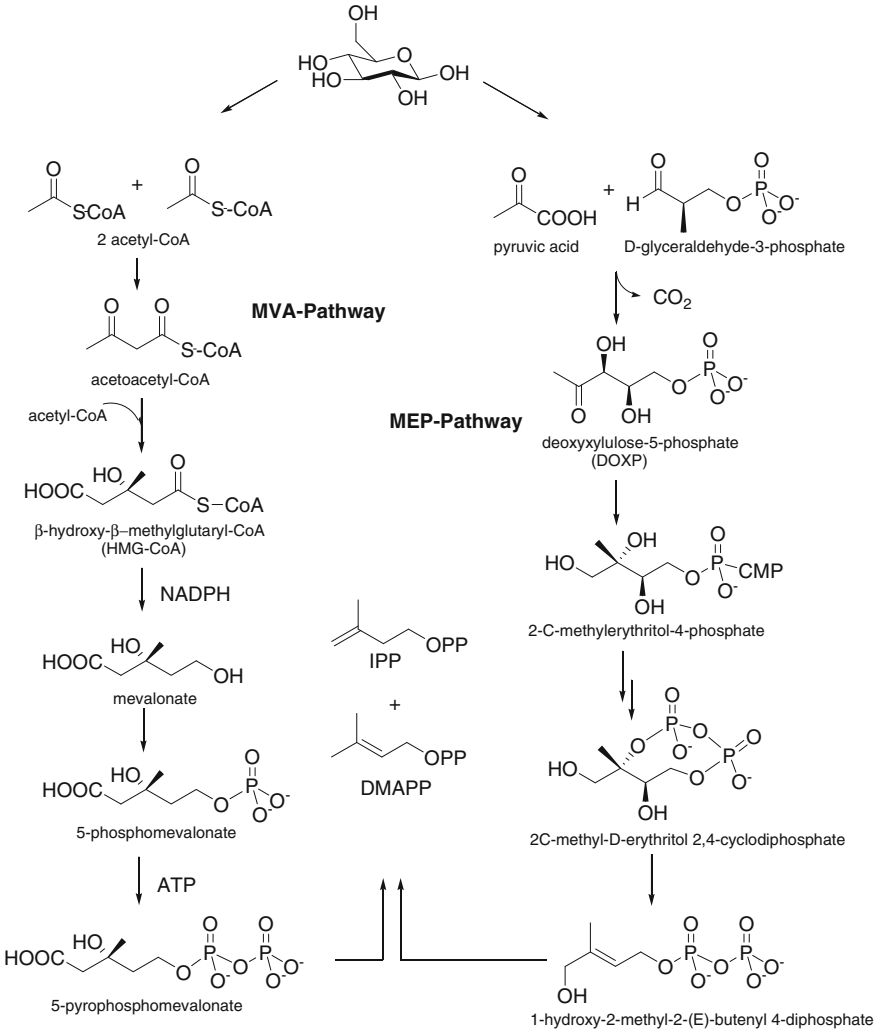

Fig. 2 MVA and nonmevalonate (or MEP) pathways

hydroxylation, transacetylation, stereospecific oxidation, benzoylations, epoxidation, esterification, and oxetane ring formation. In particular, hydroxylation reactions are common and are catalyzed by dedicated pathway P450 enzymes [60].

In step with other early efforts in the heterologous production of isoprenoid compounds, *E. coli* was a first option in efforts to generate Taxol intermediates [61, 62]. The choice to use *E. coli* coincided with the technical convenience associated with the host, namely, a rapid growth rate and well-established molecular biology protocols. The host natively utilizes the MEP pathway for cellular isoprenoid production purposes that include tRNA prenylation and steps in cell-wall

and quinone formation [63]. As such, this intrinsic support pathway was utilized to provide the universal IPP and DMAPP substrates for heterologous biosynthesis. Pathway genes associated with bottleneck steps in precursor support were identified as *dxs*, *idi*, *ispD*, and *ispF* and have since been the targets of many engineering efforts (including those of Taxol heterologous biosynthesis attempts) to improve intracellular precursor availability [64, 65].

Building upon contemporary work to generate isoprenoid compounds through *E. coli* in the 1990s [66–68], Croteau's group attempted to generate the first dedicated intermediate of the Taxol pathway, taxadiene [48, 51, 62, 69]. Given the Croteau group's pioneering work in Taxol pathway elucidation, the putative genetic material to support heterologous biosynthesis was available to test in the context of initial gene expression design. Interestingly, as advanced as these attempts were, success was only realized upon manipulation of the *E. coli* MEP pathway to improve precursor support by targeting bottleneck steps (introduced above) [70]. Even so, taxadiene titer levels achieved were modest (1.3 mg/L) [62]. Furthermore, little progress was made in generating sufficient quantities of later pathway intermediates when using *E. coli* prior to renewed efforts that began in the middle 2000s. By this time, numerous and high-titer examples of isoprenoid heterologous biosynthesis had been established through *E. coli* [71, 72]. Based upon this renewed level of success and emerging synthetic biology and metabolic engineering experimental tools, a more concerted effort was made in establishing early Taxol pathway biosynthesis through *E. coli* [61, 73, 74].

First, optimized strains designed for ample precursor supply were used as a foundation for introducing the Taxol biosynthetic pathway. Next, knowledge accumulated in gene expression design allowed successful activity from the plant-derived downstream enzymes needed to generate taxadiene and the subsequent hydroxylated intermediate. When combined, improved or new production titers of each intermediate were recorded. From this point, production was next optimized through a careful balancing of the upstream precursor support and downstream biosynthetic components of the cellular design. The final optimized product titers were further boosted through the application of small-scale bioreactor strategies [61].

Production of Taxol intermediates (and isoprenoids more generally) was also extended to yeast systems that natively possess the MVA pathway. Regarding Taxol, a strong motivation for pursuing an alternative heterologous host was the multiple steps requiring P450 hydroxylation [59, 75]. Although a bacterial host provides convenience in genetic manipulation steps, the eukaryotic nature of the yeast host provides a better cellular match to the native plant cellular environment for the Taxol biosynthetic pathway [76, 77]. As such, support mechanisms, especially in the context of the P450 enzymes, were available in this surrogate host. For example, the yeast cell could natively provide a required P450 reductase to assist in the hydroxylation reactions associated with the Taxol P450 biosynthetic steps [78]. The cellular structure of the yeast system also provides physical localization of P450 enzymes (to the endoplasmic reticulum) [79]. Hence, an emerging trend in the heterologous production of isoprenoid compounds requiring P450 activity is the use of yeast systems [77, 80, 81]. In the context of Taxol efforts, both the Croteau and

Jennewein groups tested production using yeast with successful biosynthesis of the second dedicated Taxol pathway intermediate, which required the activity of a P450 enzyme [75, 78, 82].

Finally, another potential option for microbial-based Taxol production is the use of fungal organisms. This approach is supported by interesting observations of Taxol production by endophytic fungi associated with Taxol-producing plants [83]. Several follow-up studies examine the capabilities of these organisms to produce Taxol; however, production levels were not high enough, relative to competing technology, to support production processes [84]. A recent study has also questioned endophytic fungal biosynthesis altogether, based upon the lack of compelling biosynthetic sequence identification [85]. Still, the results and observations associated with Taxol-producing fungi support the prospect of production through industrially relevant and continually developing fungal host systems such as *Aspergillus niger*, *A. nidulans*, *A. oryzae*, and *A. terreus* [86, 87].

2.5 Engineering Across Host Systems

Engineering strategies have been developed continually for Taxol biosynthetic production. Strategies vary depending on the host system and the objective. In some cases, improvements associated with final compound production have been initiated towards immediate benefits in the form of more economical and timely production routes. Longer-term research efforts, heavily featuring microbial hosts, have been dedicated to heterologous biosynthesis and post-reconstitution optimization.

In plant systems, several process engineering strategies have been summarized in Table 1. This has been the primary avenue to improving and optimizing production levels to this stage. However, more recent developments have opened the possibility of cellular-based engineering. For example, *Taxus* culture transformation methods have recently been established [88, 89]. Such advances are necessary for themes in genetic and metabolic engineering to commence. In this regard, direct pathway engineering has been initiated via overexpression of the *txs* and *dbat* biosynthetic genes coding for taxadiene synthase and 10-deacetylbaccatin III-10 β-O-acetyltransferase, respectively, in transgenic *T. chinesis*, resulting in increased production titers [90].

Engineering within microbial cells can be subdivided across different scales: genetic, metabolic, and process. The genetic level requires close attention to the details used in establishing active gene expression and the pathways responsible for biosynthesis. Viewing the wild-type cell as the basis, any engineering completed regarding gene expression must account for intrinsic challenges and resulting problems that may exist as a result of the manipulations made. Gene expression must be designed to perturb native metabolism minimally. At the same time, tuning gene expression to maximize an alternative objective relative to the cellular status quo is clearly important. Thus, the gene expression process, and the growing

number of tools available to program expression, must be designed to find an optimum of the overall objective. Key components of the process include the following.

2.5.1 Gene Source

Genetic content information (i.e., the gene sequence) must be available to initiate the design process. The native source genomic material is the obvious location for obtaining such information. Especially relevant to genes that must be introduced heterologously, the content of the gene reading frame may experience significant expression problems within a new host. This issue can be addressed by codon optimizing the gene of interest or introducing rare tRNA molecules to the new host. These considerations and others related to proper gene expression design are built into the plan of microbial biosynthesis using established and emerging tools of genetic engineering and synthetic biology [91]. The reconfigured gene or genes required for biosynthesis then provide the template for the remaining steps in pathway reconstitution.

2.5.2 Gene Expression

The gene expression process can be influenced at multiple levels. First, there is now a wealth of promoters, repressors, terminators, and other expression parameters available to design and tune gene expression (together with emerging analytical technologies to measure the resulting gene expression finely) [92]. These parameters can be combined with gene copy number and process conditions (such as temperature modulation) to optimize expression of a particular gene, which will limit detrimental metabolic imbalance. Similarly, microbial systems offer the opportunity for gene expression format variation in the way of operon designs, which offer an alternative means of modulating the potential for expression burden.

2.5.3 Expression Duration

Expression duration will also influence overall production potential. Linked to mRNA levels, extended gene expression will be closely correlated with production and will be influenced by some of the same parameters introduced above. For example, promoter type and strength will be a key determinant of mRNA content. As one example, the powerful T7 promoter has been used in several heterologous production efforts because of the strong and processive nature of the associated T7 RNA polymerase that has been built into certain microbial strains [93, 94]. Alternatively, weaker promoters stand as alternatives, as do copy number formats, to modify transcript levels [61].

Another option in transcriptional influence is the type of expression regulation. Numerous microbial systems are inducible with the *lac*, *tet*, and *ara* operators [95, 96]. Constitutive or continuous expression is the counter option to inducible systems and offers a steadier level of mRNA over time [97]. The choice of using one system versus the other is driven by experience and the specific goals of the production process. It may be desirable to attempt to decouple biosynthesis from cellular growth. In this case, an inducible expression design will allow the potential to delay the biosynthetic process until a suitable cell density has been achieved. However, in this format, inducible expression may then completely imbalance metabolism and shorten the overall production timeframe; whereas, a more measured level of constitutive expression may allow continuous and extended production while minimizing metabolic burden, potentially leading to improved production metrics. It may be difficult to choose correctly between these two expression formats in the initial design phase, but heuristics such as the likelihood of heterologous pathway products contributing to detrimental cellular effects would suggest an option of inducible expression. Doing so would allow a balance of robust biomass accumulation and detrimental side effects of biosynthesis.

The transcriptional approaches indicated above relate to mRNA generation, but influencing degradation patterns also provides a means of stabilizing transcript levels and expression over time. Particularly in bacteria, mRNA decay rates can differ by two orders of magnitude, thus, posttranscriptional processing plays a key role in controlling gene expression in cases not limited by ribosome availability [98]. For example, by introducing DNA cassettes encoding mRNA secondary structures, carotenoid biosynthesis could be modulated, resulting in target compound yield increases [99]. In addition, varying the distance between promoter and gene initiation was implicated as another influence on transcript levels and overall isoprenoid biosynthesis [100].

Finally, it should be mentioned that the majority of genetic systems indicated above were not designed with optimal pathway reconstitution in mind. Instead, most derive from attempts at single gene overexpression for the purpose of purification and characterization of the final protein product (usually in the context of biochemical characterization or protein structural determination). Thus, such systems will most likely not provide sustained and measured gene expression and subsequent product formation. More recent efforts have begun, however, to reconfigure traditional expression formats for multistep biosynthetic pathways. Commercial examples include the pET–Duet vectors from Novagen and additional plasmids designed for pathway reconstruction [101]. In other approaches, pathways are constructed using recombination-based assembly methods [102–105].

As indicated above, gene expression design is intrinsically linked to metabolic analysis and engineering. The development and continued maturity in characterization strategies, especially with regard to comprehensive and omic-type analyses, is capable of providing a complete readout of cellular states [106, 107]. With such information comes a clearer understanding of potential imbalances associated with production efforts. As indicated, such problems can be addressed at a pathway level through genetic design. However, pathway efforts must be viewed within the context

of complete metabolism. Towards this end, computational metabolic engineering has proven beneficial in predicting and identifying interactions between native and engineered metabolisms that provide the basis for additional genetic engineering for improving overall production [108, 109]. For example, stoichiometric constraint-based modeling has been utilized in the context of predicting those native reactions contributing to or detracting from the flow of carbon and other metabolites required for optimal pathway operation [110, 111]. These predictions can then be readily incorporated through the utilization of the molecular biology protocols associated with model organisms [74, 112]. It must be emphasized that the hosts must be well-characterized with regard to genome sequencing and annotation and possess tractable genetic manipulation methods. If these conditions are met, the utilization of metabolic modeling can be readily implemented in cellular-based production efforts. As an alternative to metabolic modeling, the same characterization strategies that may indicate metabolic imbalance could be used in a comprehensive fashion to allow genetic alterations in a purely experimental format. However, such efforts may be prohibitively resource intensive, which is a significant reason why cost-effective modeling approaches have found widespread utilization.

Apart from the directed metabolic engineering strategies indicated above, there also exist nondirected efforts that take advantage of classical biological principles of perturbation and selection. For example, native production of various natural products have effectively utilized mutagenesis and screening protocols [113]. In particular, such approaches are viable options when production hosts are not well-characterized or genetically tractable. In such cases, genetic manipulations towards improved phenotypes are possible, but the genotypic changes made are not done so in a directed fashion and, therefore, there is less of an engineering aspect to these approaches. Nonetheless, they have been utilized with great success with several natural products [114–119].

Finally, at the process level, there exist additional engineering options to improve final production metrics further. First, there is the opportunity to scale production conditions from culture tubes and shake flasks to well-controlled bioreactors that allow precise maintenance of parameters such as pH, temperature, agitation, and dissolved oxygen content in addition to larger reaction volumes [120]. This improvement in process control and overall scale typically translates to improved final production titers [121, 122]. However, the advantage to utilizing nonaggregating, unicellular microbial hosts possessing simple media requirements and rapid growth kinetics has the potential to modify the typical batch bioreactor configuration to include a nutrient feed capable of greatly extending volumetric cell density and productivity. In this format, a highly concentrated nutrient stream is slowly fed to the primary culture, allowing for a finely controlled and extended growth profile and eliminating the carbon nutrient limitations that would normally accompany a batch reactor [123–125]. The fed-batch design therefore allows for effective cell density scaling with a modest increase in total volume, effectively allowing continued scaling of production without the need physically to increase the size of the bioreactor.

Although a fed-batch bioreactor design will eliminate carbon source limitation, there exists the danger of oxygen limitation. This concern can be addressed through the reconfiguration of bioreactor air ports or the introduction of pure oxygen [126, 127]. Related to nutrient input and other process parameters, the process production formats can also be optimized utilizing design of experiments (DOE) techniques [128]. In certain situations, such as media optimization, the approach may be best accomplished utilizing small-scale (i.e., 96-well) culture formats with subsequent scaled experiments used to confirm results [129, 130]. Alternatively, the same sort of optimization experiments could be applied to the controllable process parameters at the bioreactor scale [131].

3 Summary, Conclusions, and Outlook

Current Taxol production processes utilize plant sources in either semisynthetic or culture-based formats. Such routes have allowed for the distribution and medicinal use of this potent anticancer compound. However, relative advantages of microbial production hosts, together with emerging technology and examples of heterologously produced isoprenoid compounds, have initiated studies of reconstituted production efforts through host systems such as *E. coli* and *S. cerevisiae*. Regardless of the biological production host, engineering strategies have been implemented across several scales.

Plant-based production has seen a transition from direct (and nonsustainable) extraction from the native Pacific yew tree to industrial production dependent upon plant cell culture. Bridging these two production strategies was a semisynthetic route that first allowed larger-scale access to the Taxol compound. As more information and technology are gathered using plant-cell culture, the application of metabolic and process engineering is expected to continue to result in improved titers. However, the challenge of engineering plant cells has spurred parallel research in heterologous biosynthesis.

Engineering of microbial production hosts has been focused on two goals. First, there is the need to implement fully and reconstitute successfully the complete Taxol pathway (or enough of the pathway to allow semisynthesis). A number of genetic engineering strategies have been utilized to enable early-stage pathway intermediate production. These same tools stand as a way to enable the second objective which is to design production carefully for the purpose of streamlining later metabolic and process engineering. Combined, the available of emerging engineering options associated with the genetic, metabolic, and process scales will each need to be utilized and optimized to make compounds such as Taxol feasible for microbial production with the promise of even more widespread accessibility to this and similar compounds.

This vision is therefore a key future objective. Furthermore, successful Taxol heterologous biosynthesis would allow an additional frontier: the potential to engineer rationally the biosynthetic steps for molecular diversification. The goals

associated with molecular diversification could range from improving the formulation and solubility of the Taxol compound to designing altered anticancer (and other forms of) bioactivity [132, 133].

The same themes summarized above for Taxol provide the basis for a broader future direction. Namely, a highly desired vision is to achieve the successful production, heterologous reconstitution, and manipulation of numerous other isoprenoid natural products, including those yet to be discovered. The success recorded thus far regarding the medicinal utility of isoprenoids represents only a small fraction of the possibilities available through nature. This chapter and ongoing work are dedicated to summarizing and projecting opportunities to identifying, producing, and benefiting from similar production efforts with increasingly successful frequency.

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