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Recent Advances in the Biosynthesis and Biotechnological Production of Taxol

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ABSTRACT

Taxol (paclitaxel), a widely used chemotherapeutic agent, was originally isolated from the bark of *Taxus brevifolia*. However, its extremely low natural abundance has prompted the development of alternative production methods. Currently, the predominant commercial strategy relies on semi-synthesis from a more abundant precursor, 10-deacetyl-baccatin III, extracted from the needles of various *Taxus* species. Advances in synthetic biology are opening promising new avenues for more sustainable and scalable Taxol production. Recent progress, including the discovery of a nuclear transport factor 2 (NTF2)-like protein FoTO1, the characterization of a series of oxetane-forming P450 enzymes, and the identification of taxoid-2′-oxoglutarate-dependent dioxygenase (T2′OGD) and taxoid-3′-*N*-benzoyl-transferase (T3′NBT) responsible for C2′α-hydroxylation and 3′-*N* benzoylation, has greatly advanced our understanding of the Taxol biosynthetic pathway and enabled its reconstitution in both plant and microbial hosts. In this review, we summarize recent progress in defining the Taxol biosynthetic pathway and highlight synthetic biology strategies with the greatest potential to support reliable and sustainable production of this essential anticancer agent.

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1. Introduction

Taxol (paclitaxel) stands as one of the most successful natural product-derived drugs in medical history and remains a cornerstone of modern chemotherapy across a wide range of malignancies. First isolated from the bark of *Taxus brevifolia* (*T. brevifolia*; Pacific yew) in 1971, Taxol was found to possess potent antileukemic and tumor-inhibitory properties [1]. Mechanistic studies revealed that Taxol functions as a microtubule-stabilizing agent *in vitro* [2,3]. Taxol binds to a specific site on the inner surface of β-tubulin [4], and thus promotes tubulin polymerization while simultaneously inhibiting depolymerization, thereby disrupting normal microtubule dynamics. As a result, Taxol induces mitotic arrest and activates apoptotic pathways. Owing to its clinical efficacy, Taxol was approved by the US Food and Drug Administration (FDA) in 1992 for the treatment of ovarian cancer, a major health burden among women [5].

Despite its remarkable anticancer efficacy, the sustainable production of Taxol has long been constrained by its natural source—*Taxus* plants, which are slow-growing conifers with restricted ecological distribution and inherently low metabolite yields. Harvesting Taxol from the bark is not only inefficient but also ecologically destructive [6]. These limitations, coupled with the compound's intricate chemical structure, have made Taxol a long-standing challenge in synthetic chemistry. Over the past four decades, extensive efforts have been made to achieve total chemical synthesis of Taxol [7–11]. In addition, a semi-synthetic strategy was developed, which now accounts for the majority of industrial Taxol production. [12]. This approach utilizes more abundant and accessible precursors, such as 10-deacetyl-baccatin III or baccatin III, extracted from the needles of cultivated *Taxus* plants without compromising their viability. These precursors undergo chemical coupling with a synthetically assembled C13 side chain to yield Taxol.

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This approach substantially improves yields while reducing environmental impact, providing a more economically and ecologically sustainable alternative.

Synthetic biology has recently emerged as a transformative strategy for the sustainable and scalable production of complex plant-derived natural products. By reconstructing and optimizing biosynthetic pathways within engineered plant-based or microbial chassis, this approach enables controlled biosynthesis in renewable platforms, circumventing the limitations of traditional extraction and chemical synthesis methods. Synthetic biology has already shown promise in the biosynthesis of several medicinal molecules, such as opioids [13], cannabinoids [14], celastrol [15], and vinblastine [16]. However, its application to Taxol has been delayed by gaps in our understanding of its complete biosynthetic pathway.

Recent advances in *Taxus* genomics, including the release of high-quality genomes for two Taxol-producing species (*Taxus chinensis* and *Taxus wallichiana*), have substantially accelerated research on Taxol biosynthesis [17,18]. Together with comprehensive transcriptomic datasets and integrated multi-omics resources, these advantages have greatly expanded our capacity to identify and functionally characterize the enzymes involved in this highly complex diterpenoid pathway. Leveraging these tools, several recent breakthroughs lifted key barriers in our understanding of Taxol biosynthesis. These include the elucidation of the oxetane ring-forming mechanism (2023–2024) [19–22], the identification of FoTO1, a nuclear transport factor 2 (NTF2)-like protein essential for Taxol biosynthesis (2025) [23], and the discovery of final biosynthetic steps such as C2′α hydroxylation and 3′-*N* benzoylation (2025) [24]. These findings have not only deepened our understanding of the biosynthetic mechanism of this highly complex molecule but also created new opportunities for pathway engineering. As a result, attention has increasingly turned to the development of engineered cell factories for Taxol biosynthesis [25]. This innovative approach has the potential to revolutionize conventional production methods that rely heavily on plant resources. So far, significant progress has been made in reconstructing parts of the biosynthetic pathway in heterologous systems, including *Nicotiana benthamiana* (*N. benthamiana*; hereafter tobacco), *Escherichia coli* (*E. coli*), *Saccharomyces cerevisiae* (*S. cerevisiae*; hereafter yeast), and cyanobacteria [19,21,22,26–28]. These systems have successfully produced key intermediates and demonstrated proof-of-concept for scalable bioproduction of Taxol.

In this review, we summarize recent advances in Taxol biosynthesis, with a particular focus on synthetic biology approaches that promise to enable sustainable and scalable production of this vital anticancer agent.

2. Progress in deciphering Taxol biosynthesis

The Taxol biosynthetic pathway can be broadly divided into three distinct stages (Fig. 1): ① formation of the taxane core structure, primarily through the cyclization of the universal diterpene precursor geranylgeranyl diphosphate (GGPP) to taxadiene; ② a series of subsequent oxidative and acylation modifications that decorate the taxadiene backbone to yield the advanced intermediate baccatin III; and ③ the final steps involving the attachment of the β-phenyalalnoyl chain to the C13 hydroxyl group of baccatin III, followed by additional modifications of the side chain to produce the active compound, Taxol.

Stage 1: The taxadiene backbone formation. The first committed step in Taxol biosynthesis is catalyzed by taxadiene synthase (TXS), which converts GGPP into taxa-4(5),11(12)-diene (**1**; endotaxadiene) (Fig. 2) [29]. This reaction initiates the formation of the taxane core. TXS was first isolated from the stems of *T. brevifolia* [30] and has since been extensively characterized. Notably, except for endotaxadiene, a minor isomeric product, taxa-4(20),11(12)-diene (exotaxadiene, **2**), accounting for ~5% of the total product, was also identified. The predominant formation of endotaxadiene was unexpected, given that taxanes in yew commonly contain 4(20)- and 11(12)-double bonds [31], exotaxadiene had initially been hypothesized as the native precursor [32,33]. In 2011, the X-ray crystal structure of TXS was resolved [34], revealing a modular architecture composed of three α-helical domains.

Stage 2: Core oxidative decorations on the taxadiene backbone. The formation of the core taxane scaffold in Taxol entails a series of oxidative modifications to the C20 hydrocarbon backbone. [35]. While heterologous expression systems have enabled the efficient production of taxa-4(5),11(12)-diene, progress toward full pathway reconstitution has been hampered by the complexity of downstream modifications. Between 1996 and 2004, several cytochrome P450 enzymes (CYPs) with activity on taxane intermediates were identified, such as 2α-, 5α-, 7β-, 10β-, and 13α-hydroxylases [36–41]; however, their specific roles, order of action, and native interactions remained largely unresolved.

Since its identification in 1996, the cytochrome P450 taxane 5α-hydroxylase (T5αH; CYP725A4) has been widely thought as the enzyme catalyzing the first oxidative transformation in Taxol biosynthesis. This reaction hydroxylates the taxane core and induces a double-bond shift to generate taxadien-5α-ol (**3**). Alongside this primary product, T5αH also produces large amounts of side products such as 5(12)-oxa-3(11)-cyclotaxane (OCT) and 5(11)-oxa-3(11)-cyclotaxane (iso-OCT) in both *in vitro* and *in vivo* systems [36]. Notably, these side products have not been detected in any *Taxus* species, highlighting a significant discrepancy between heterologous expression systems and the native biosynthetic context.

As expression of T5αH in various microbial hosts has consistently resulted in low yields of **3** and a predominance of unproductive side products (OCT and iso-OCT), reconstruction of the proposed downstream steps of the pathway was significantly hindered. Kinetic studies by Jennewein et al. [33] provided further insight into the enzyme's substrate specificity, revealing that T5αH binds exotaxadiene with nearly twice the affinity compared to endotaxadiene, with equilibrium binding constants (K_s) of 3–5 μmol·L^{-1} and 5–8 μmol·L^{-1}, respectively. These findings suggested that exotaxadiene may be the more physiologically relevant substrate for T5αH. However, attempts to engineer TXS to increase the proportion of exotaxadiene in order to improve the specificity of T5αH oxidation have so far achieved limited success [42].

In a breakthrough study employing a multiplexed perturbation strategy to identify previously uncharacterized genes, McClune and colleagues [23] identified FoTO1 as a critical regulatory component. Expression of FoTO1 in engineered tobacco enhanced flux through the early Taxol pathway, increasing production of the first committed intermediate, **3**, by 10- to 17-fold. These findings support a model in which FoTO1 acts as a metabolic scaffold, enhancing the specificity of downstream reactions and directing the activity of T5αH toward the formation of the desired product (**3**), while suppressing the generation of undesired side products such as OCT. Co-expression and epitope-tagging experiments, followed by immunoprecipitation, revealed a physical interaction between FoTO1 and T5αH, further supporting its role as a scaffolding or accessory protein that facilitates the assembly or stabilization of early enzyme complexes. Moreover, FoTO1 was also shown to enhance the catalytic efficiency of taxane 13α-hydroxylase (T13αH; CYP725A2), a bifunctional P450 enzyme capable of oxidizing both the 5α and 13α positions. Further experiments suggested that, in the presence of FoTO1, T13αH is sufficient to support the synthesis of taxadien-5α,13α-diol (**6**) in the absence of T5αH, revealing a functional redundancy at the first two steps of the

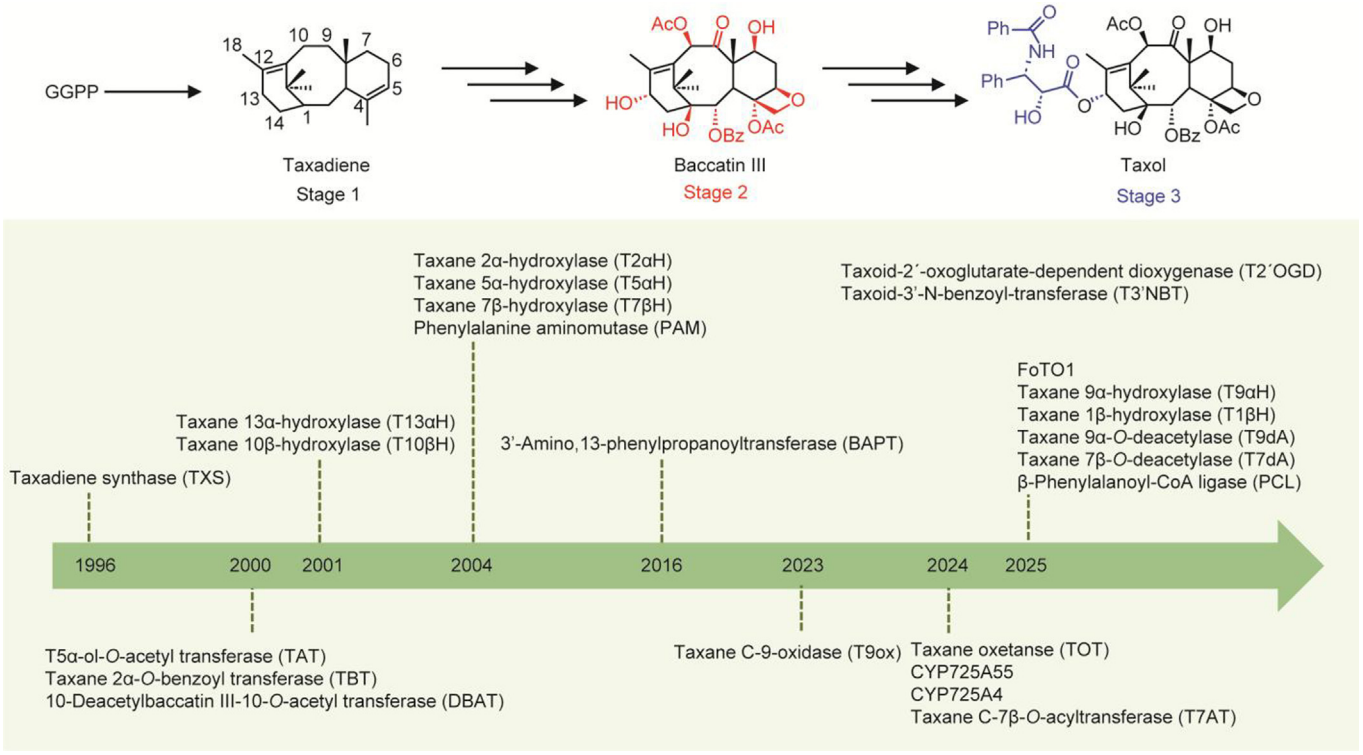


Fig. 1. Timetable of Taxol biosynthetic pathway elucidation.

pathway [23]. This functional overlap allows one gene (either *T5αH* or *T13αH*) to serve as a backup, compensating for the loss of function in the other, thereby ensuring pathway resilience and sustained taxoids production [43]. Additionally, their differential expression across distinct cell types enables the synthesis of taxoids lacking C13 oxidation, contributing to the structural diversification of taxoids and the expansion of related metabolic branches.

The next step is thought to be the acetylation of the **3** to taxadien-5α-ol acetate (**4**), by the enzyme T5α-ol-*O*-acetyltransferase (TAT). This step is followed by the oxidation and acetylation of the C10 and C13 positions by taxane 10β-hydroxylase (T10βH), T13αH, and 10-deacetyl-baccatin III-10-*O*-acetyltransferase (DBAT), respectively, leading to the formation of 5,10-diacetoxy-13-hydroxy-taxadiene (**9**). Notably, an alternative route from **6** to **9** may also exist in planta, bypassing T5αH, due to the aforementioned functional redundancy of T13αH and T5αH (Fig. 2).

The McClune et al. [23] illuminated the next step, revealing the functional diversity of taxane 9α-hydroxylases (T9αH). T9αH-750C, a member of the CYP750C family, was shown to catalyze C-9 hydroxylation. Strikingly, it shares less than 20% sequence identity with previously characterized CYP725A family enzymes (T9αH-725A), which were believed to catalyze the same reaction based on their ability to produce the C9-hydroxylated taxanes taxusin and 9,10-deacetyl taxusin [19,21,26]. Notably, T9αH-750C and T9αH-725A appear to exhibit distinct substrate preferences: the former favors C-13α-hydroxylated taxanes, while the latter prefers C-13α-acetoxylated taxanes, suggesting that T9αH activity evolved independently in these two P450 lineages. These differences in sequence and substrate specificity imply that T9αH-750C and T9αH-725A operate in separate branches of the Taxol biosynthetic pathway, leading to the formation of C-13α-hydroxylated and C-13α-acetoxylated taxanes, respectively.

Following the synthesis of 5,10-diacetoxy-9,13-dihydroxy-taxadiene (**10**), TAT acetylates the 9α hydroxy position to yield the triacetylated intermediate **11**, in a reaction that appears to diverge from the expected route, as the 9α position is not acetylated in taxol. This acetyl group performs a protective function, being removed later in the pathway. This step is succeeded by the concerted hydroxylation and benzoylation of C2 to yield **13**, by the previously discovered taxane 2α-hydroxylase (T2αH) and taxane 2α-*O*-benzoyltransferase (TBT) [40,44]. In the next two biosynthetic steps, **13** is hydroxylated at position 7β by taxane 7β-hydroxylase (T7βH) and acetylated at the same position by taxane C-7β-*O*-acetyltransferase (T7AT). This acetyltransferase was independently identified by two research groups, and its function was verified in both yeast and tobacco expression systems [21,23]. Although the 7β-*O*-acetyl group is absent in Taxol and its direct precursor baccatin III, it has been shown to be essential for the formation of the oxetane ring in later stages of the biosynthetic pathway [23].

The mechanism leading to the formation of the oxetane ring of taxol has been extensively debated, and at least three routes have been proposed. Initially, Zhang and colleagues [26] proposed that this is driven by a 2-oxoglutarate/Fe(II)-dependent dioxygenase (2-ODD). This enzyme was proposed to mediate epoxidation of the C4–C20 double bond, based on its observed activity in catalyzing 4,20-epoxidation of taxusin. However, no direct evidence supporting this function was provided, as the structures of the three enzymatic products formed from taxadiene could not be elucidated by nuclear magnetic resonance (NMR) spectroscopy. Moreover, *in vitro* characterization of the enzyme's activity was hindered by the inability to obtain an active form of the enzyme through heterologous expression in yeast or bacteria. Subsequently, prompted by the identification of two naturally occurring taxanes, 1β-dehydroxy-4α-deacetylbaccatin IV and 4-deacetylbaccatin IV, that contain unacetylated oxetane rings [45,46], Zhao and colleagues [22] proposed that acetylation may occur after the oxetane ring has already formed. Further supporting this shift in understanding, Ondari and Walker [47]

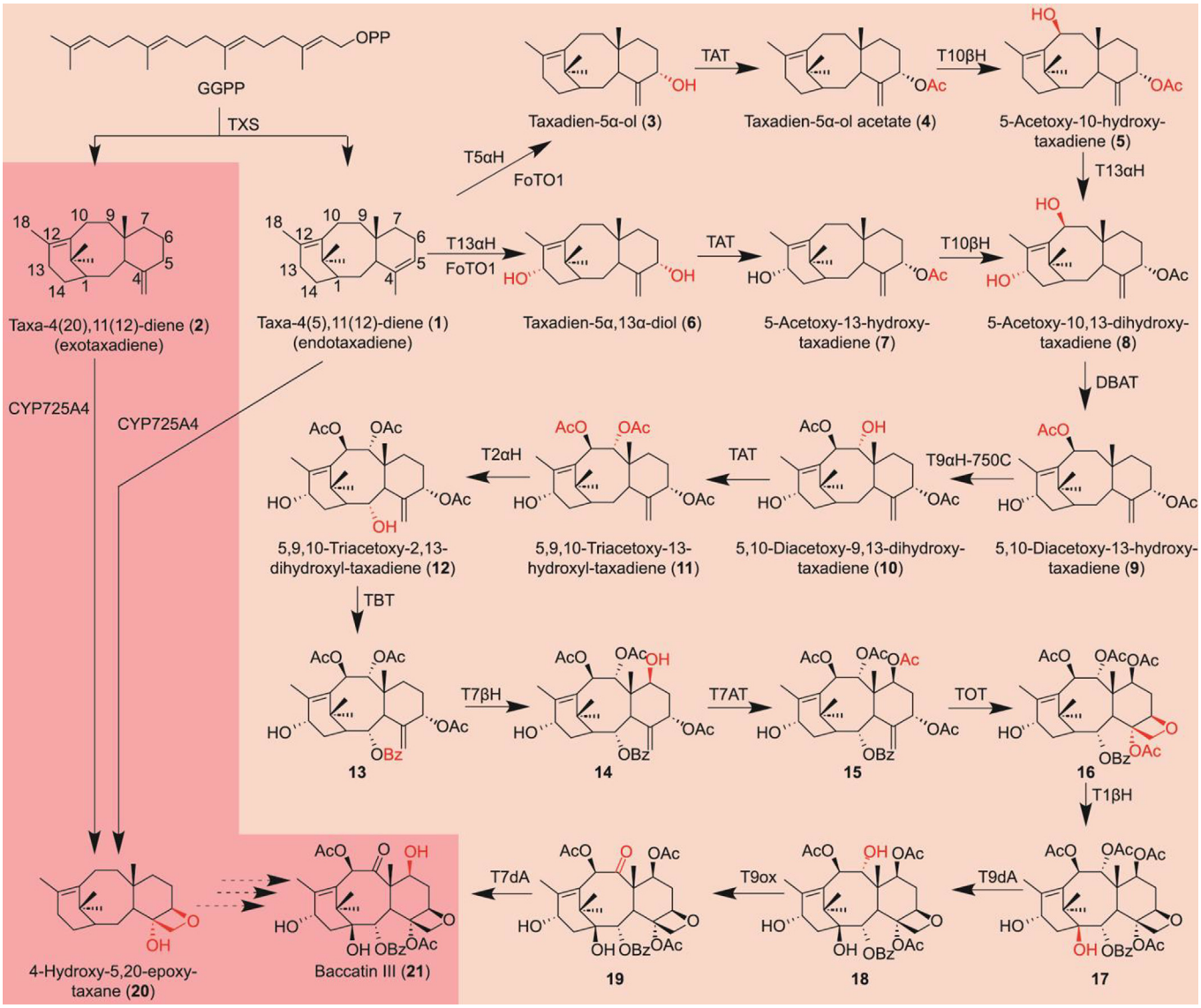


Fig. 2. Overview of the Taxol biosynthetic pathway leading to the key intermediate baccatin III (stages 1 and 2). OPP: pyrophosphate group.

demonstrated through *in vitro* assays that a promiscuous acetyltransferase taxane C-10β-*O*-acetyl transferase (T10AT), can acetylate oxetane-containing taxanes that are deacetylated at the C4 position, reinforcing the idea that acetylation does not precede oxetane ring formation. Building on these observations, Zhao et al. [22] proposed an alternative pathway in which CYPs mediate the formation of the oxetane ring through oxidation events that precede C5 acetylation. To test this hypothesis, they analyzed the genomic and transcriptomic data from various *Taxus* species to identify potential cytochrome P450 candidates. Functional characterization of these enzymes in two independent systems (yeast and tobacco) revealed that CYP725A4, the same enzyme responsible for the formation of **3**, can catalyze two consecutive epoxidation reactions that ultimately lead to the formation of the oxetane ring. Remarkably, both endotaxadiene and exotaxadiene were identified as substrates for this reaction, suggesting the presence of multiple, alternative biosynthetic routes within the Taxol pathway.

In parallel to Zhao's discovery [22], several other studies have also elucidated the formation of the oxetane ring through different mechanisms. Jiang et al. [19] identified taxane oxetanase 1 (TOT1), a member of the CYP725A subfamily from *Taxus mairei* (*T. mairei*), as a bifunctional oxygenase capable of directly converting the olefin moiety of 5α-acetylated taxadiene into both epoxide and an oxetane ring. However, their findings indicated that TOT1 does not function as an isomerase to convert an epoxide intermediate into the oxetane ring. Based on this, they proposed that epoxidation is not a necessary intermediate in oxetane ring formation. A similar discovery was made by Li et al. [20], who identified a TOT1 homolog (*Tm*CYP1) from *Taxus* × *media* cell cultures. *Tm*CYP1 catalyzed the direct formation of the oxetane ring from 5α-acetylated taxadiene, but it did not recognize **3** as a substrate. Through substrate feeding and isotope labeling (^{2}H and ^{18}O), they proposed a mechanism involving intramolecular oxidation-acetoxy rearrangement, suggesting that oxetane ring formation occurs via a concerted process. Their results further indicated that the 4,20-epoxide might be a side product rather than an essential intermediate and emphasized the crucial role of the C5-*O*-acetyl moiety in oxetane formation. Supporting this hypothesis, a third independent study by Yang et al. [21] utilized C5-acetyl-^{18}O-labeled 2α-benzoyloxy-7β-acetoxytaxusin as a substrate, which was introduced into *S. cerevisiae* expressing a CYP725 family enzyme (another TOT1 homolog) from *Taxus yunnanensis*. Their

results demonstrated that oxetane formation occurs through a direct oxidation-acyl rearrangement mechanism, without involvement of an epoxide ester intermediate.

Following the discovery of TOT1 as the enzyme catalyzing the conversion of **15** to **16**, two oxoglutarate-dependent dioxygenases (OGDs), 2-ODD184 and 2-ODD686, were identified as taxane 1β-hydroxylases (T1βH), responsible for the next step in the pathway [23]. Their functions were confirmed through co-expression with upstream pathway genes and a C-13α-*O*-acetyltransferase (TAX19), resulting in the production of baccatin VI. While both enzymes can hydroxylate the C-1 position of compound **16**, 2-ODD686 displays markedly higher catalytic efficiency and is likely the dominant isoform operating in planta.

By systematic screening of α/β-hydrolase candidates, McClune et al. [23] identified two deacetylases, DeAc898 and DeAc1023, that catalyze the stepwise removal of *O*-acetyl groups at the C-7 and C-9 positions of baccatin VI, yielding a product consistent with the 9-dihydro-13-acetylbaccatin III (9DHAB) standard. Although the precise biological roles of these additional acetylations remain inconclusive, they are likely to act as transient protecting groups during biosynthesis, potentially preventing undesired side reactions or stabilizing intermediates. This strategy mirrors protective-group chemistry observed in the biosynthesis of other plant-derived terpenoids, such as limonoids [48], suggesting that reversible acylation may represent a widespread and underappreciated regulatory mechanism in plant specialized metabolism. The discovery of these deacetylases thus not only fills a missing link in the taxane biosynthetic pathway but also highlights a broader evolutionary logic underlying specialized metabolite diversification in plants.

Finally, a putative C-9 oxidase, taxane C-9-oxidase (T9ox), was independently identified by McClune et al. [23] and Zhang et al. [26] as a member of the 2-ODD family. T9ox catalyzes the oxidation of 9DHAB to its corresponding C-9 ketone, completing a key late-stage transformation in the formation of baccatin III. Separately, Jiang et al. [19] reported a homologous cytochrome P450 enzyme, T9αH1, from *T. mairei*, which catalyzes hydroxylation at the C-9 position [19]. When co-expressed in tobacco alongside additional pathway enzymes, including T5αH, T13αH, T2αH, T7βH, TOT, TAT, and TBT, this system successfully produced baccatin III. Notably, despite the absence of characterized C-1 and C-10 hydroxylases in this reconstituted pathway, the corresponding modifications were still detected. This observation suggests that endogenous tobacco enzymes may complement these steps, or that one or more of the introduced oxygenases exhibit relaxed substrate specificity. Such enzymatic promiscuity underscores the inherent flexibility of certain oxygenases in the Taxol biosynthetic pathway and presents opportunities for pathway reconstitution and metabolic engineering in heterologous systems.

Stage 3: The late Taxol pathway: from baccatin III onwards. The final steps of Taxol biosynthesis involve the attachment of β-phenylalanine to the C13 hydroxyl group of baccatin III, followed by two additional modifications (Fig. 3). The biosynthetic pathway of converting baccatin III into Taxol contains five enzymatic steps, including isomerizing α-phenylalanine to β-phenylalanine, activating of β-phenylalanine by coenzyme A to produce β-phenylalanoyl-CoA, condensing β-phenylalanoyl-CoA to C13 position of baccatin III, oxidizing C′2 of the β-phenylalanyl side chain, and adding a benzoyl group at its 3′-*N* position (Fig. 3). Among the five steps, catalytic enzymes of two steps were established early in the efforts to elucidate the pathway. These two steps are the production of β-phenylalanine from α-phenylalanine by phenylalanine aminomutase (PAM) [49] and the condensation of phenylalanyl side chain with baccatin III by 3′-amino,13-phenylpropanoyltransferase (BAPT) [50]. The other three steps, including attaching a CoA moiety to β-phenylalanine by a CoA ligase, hydroxylating C2′ of the phenylalanyl side chain by an oxidase, and benzoylating its 3′-*N* position by a CoA-dependent transferase, have been controversial for some time. However, in the past two years, these steps have been independently elucidated by two research groups [23,24].

β-Phenylalanoyl-CoA ligase (PCL) was identified by McClune et al. [23] through the application of single-cell sequencing technology. This approach led to the discovery of AAE-867.5, an enzyme that efficiently catalyzes the CoA ligation of β-phenylalanine. This activation enables the subsequent condensation of β-phenylalanine with baccatin III to form *N*-debenzoyl-2′-deoxy-taxol [23]. Additionally, they demonstrated that the other two previously reported CoA ligases had very low, if any, activity in *N. benthamiana* [26,51]. Kampranis's lab independently reported similar findings regarding these two CoA ligases [24]. Based on these results, AAE867.5 is likely the correct CoA ligase in the Taxol biosynthetic pathway, while the other two CoA ligases were likely misidentified. This misidentification likely stemmed from the use of *in vitro* assays, which may have resulted in low-level non-specific activity [50], interference from endogenous tobacco enzymes, or reliance on indirect evidence to infer the enzymatic function of the candidate proteins [26].

C2′ hydroxylation is essential for the biological activity of Taxol [52]. For many years, this step was widely presumed to be catalyzed by a cytochrome P450 enzyme, prompting extensive efforts to screen candidate CYPs. This effort led to the identification of the cytochrome P450 enzyme TB506 [53]. However, subsequent attempts by other laboratories to replicate its reported activity were unsuccessful [23]. Continuing with the hypothesis that C2′ hydroxylation is catalyzed by a cytochrome P450 enzyme, Liang et al. [24] screened a substantial number of CYP candidates but was ultimately unsuccessful in identifying one with the desired activity. As a result, the focus of their investigation shifted to a different class of oxidases, the OGD family. Certain members of this family are known to participate in post-translational protein hydroxylation modifications [54], which involve oxidative mechanisms similar to the C2′ hydroxylation observed in *N*-debenzoyl-2′-deoxy-Taxol. By screening candidate enzymes within this family, they identified taxoid-2′-OGD (T2′OGD), an enzyme that efficiently catalyzes the C2′ hydroxylation step. The enzymatic function of T2′OGD was further validated in two independent heterologous expression systems, tobacco and yeast, providing compelling evidence that its activity is genuine and constitutes a critical step in the Taxol biosynthetic pathway [24].

The final modification in the Taxol biosynthetic pathway, 3′-*N* benzoylation, was previously thought to be catalyzed by the enzyme 3′-*N*-debenzoyl-2′-deoxy-taxol-*N*-benzoyltransferase (DBTNBT). This was based on *in vitro* assays demonstrating its ability to benzoylate the 3′-*N* position of both *N*-debenzoyl-Taxol and *N*-debenzoyl-2′-deoxy-taxol [55,56]. However, *in vivo* studies showed that DBTNBT only acts on *N*-debenzoyl-2′-deoxy-taxol, revealing a level of substrate specificity that was not evident from the earlier *in vitro* experiments [23]. Moreover, the product of this reaction, 2′-deoxy-taxol, has not been detected in any *Taxus* crude extracts. In addition, T2′OGD is unable to hydroxylate 2′-deoxy-taxol, suggesting that the 3′-*N* benzoylation of *N*-debenzoyl-taxol is catalyzed by a different benzoyltransferase. Supporting this, Liang et al. [24] identified a transferase, taxoid-3′-*N*-benzoyltransferase (T3′NBT), which specifically recognizes *N*-debenzoyl-taxol as its substrate and efficiently produces Taxol in both tobacco and yeast. Since T2′OGD cannot act on 2′-deoxy-taxol and T3′NBT does not accept *N*-debenzoyl-2′-deoxy-taxol as substrate, these findings confirm that 2′-hydroxylation precedes 3′-*N* benzoylation in the Taxol biosynthetic pathway.

Collectively, the five enzymes required to convert baccatin III into Taxol have now been fully characterized. Among these, three CoA-dependent transferases—DBAT, BAPT, and T3′NBT—exhibit

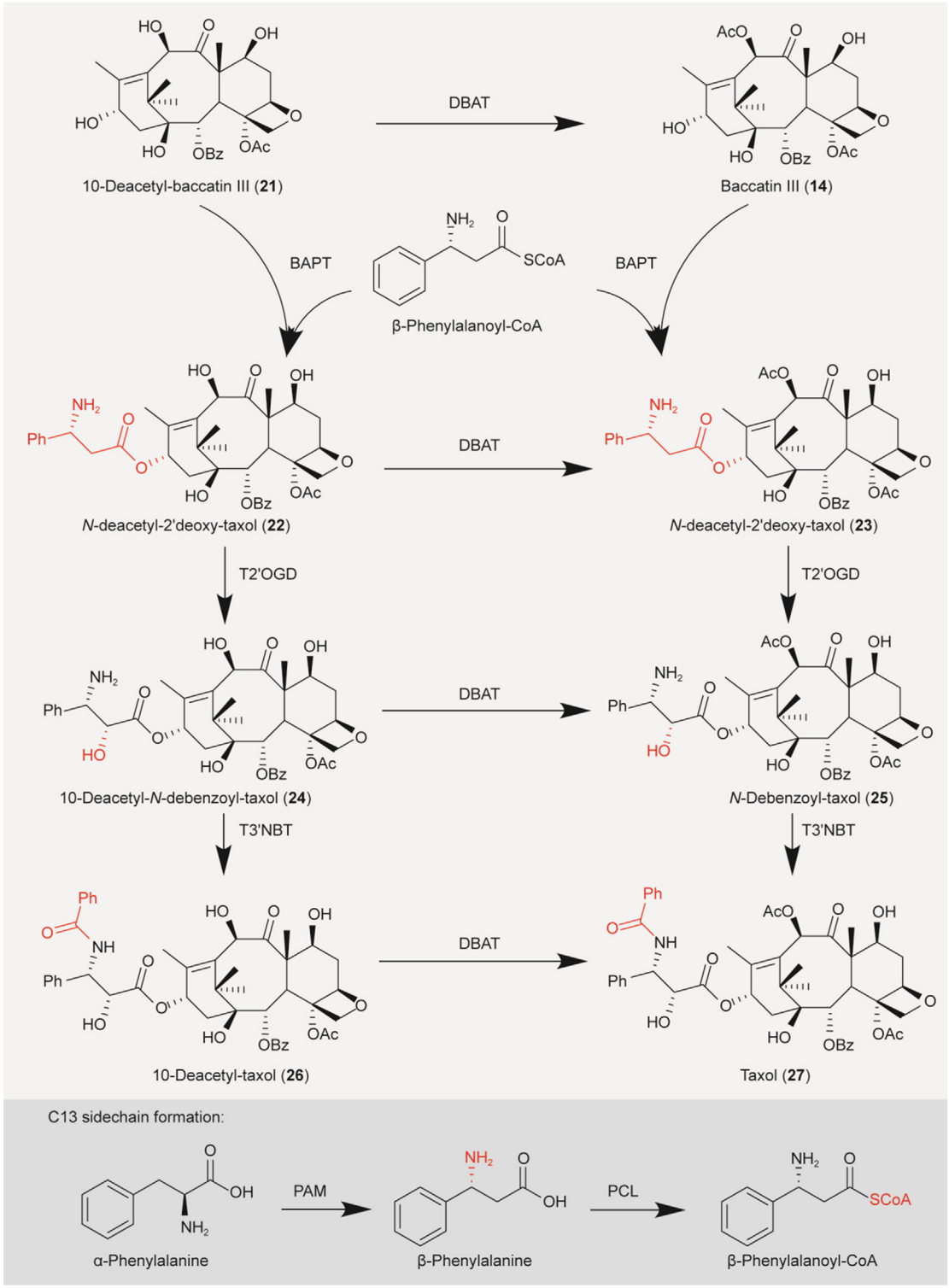


Fig. 3. Overview of the Taxol late biosynthetic pathway from 10-deacetyl-baccatin III or baccatin III to Taxol (stage 3).

notably broad substrate specificity. DBAT catalyzes acetylation at the C10 position across a range of substrates, including 5α-acetoxy, 10β, 13α-taxane-diol, 10-deacetyl-baccatin III, *N*-debenzoyl-2′-deoxy-10-deacetyl-taxol, *N*-debenzoyl-10-deacetyl-taxol, and 10-deacetyl-taxol. BAPT accepts both 10-deacetyl-baccatin III and baccatin III as substrates, while T3′NBT can benzoylate *N*-debenzoyl-Taxol as well as *N*-debenzoyl-10-deacetyl-Taxol. Importantly, both the substrates and products of these enzymes have been detected in crude *Taxus* extracts, providing strong *in vivo* support for their catalytic activities and physiological relevance. These findings indicate that the late stages of Taxol biosynthesis are not strictly linear but instead follow a grid-like, branched pathway, in which multiple intermediates can be interconverted through the action of broadly specific enzymes. This modular biosynthetic architecture may provide metabolic robustness, allowing the plant to flexibly respond to environmental or developmental cues by

channeling flux through alternative intermediates. Importantly, such enzymatic promiscuity holds significant potential for synthetic biology applications. By leveraging the relaxed substrate selectivity of key enzymes, it becomes feasible to generate novel Taxol analogs through combinatorial biosynthesis. These analogs may exhibit enhanced pharmacological profiles, reduced toxicity, or the ability to overcome drug resistance. Moreover, this grid-like logic presents valuable opportunities for pathway optimization in heterologous systems. In microbial or plant chassis, metabolic flux can be redirected through the most efficient branches by tuning enzyme expression levels, selecting high-activity isoforms, or introducing engineered variants with improved specificity. Such strategies can alleviate pathway bottlenecks, enhance precursor utilization, and ultimately improve overall yields of Taxol and its derivatives in engineered hosts.

3. Biology systems for Taxol production

Elucidation of the Taxol biosynthetic pathway has catalyzed significant advances in synthetic biology efforts aiming at establishing its sustainable and scalable bioproduction (Fig. 4). The identification of key biosynthetic enzymes, coupled with metabolic engineering in both plant and microbial systems, has enabled the heterologous production of Taxol or its key intermediates in diverse host organisms including tobacco, tomato, *E. coli*, and yeast, and thus providing a promising example for the green production of highly important natural products-based pharmaceuticals.

3.1. Taxus cell suspension cultures

Cell suspension cultures remain one of the most effective and adaptable methods for the production of Taxol and play a supplementary role compared to the dominant semi-synthetic route. Taxol production in suspension cell cultures typically involves two key phases: the establishment of stable suspension cell lines and the induction of taxane biosynthesis [57]. This phased approach is essential because the accumulation of Taxol within the cells or the surrounding medium can exert cytotoxic effects, inhibiting cell growth and metabolic activity and ultimately reducing production efficiency. The application of chemical elicitors (such as methyl jasmonate) has proven highly effective in enhancing Taxol yields [58]. In parallel, genetic engineering strategies have further advanced the potential of suspension cultures. Perez-Matas et al. [59] demonstrated that overexpression of BAPT, in combination with elicitor treatment, led to substantial increases in Taxol accumulation. With the recent elucidation of nearly the entire Taxol biosynthetic pathway, including several oxygenases, acyltransferases, and deacetylases, there is now a compelling opportunity to systematically enhance taxane production in cell cultures by targeting upstream bottlenecks.

3.2. Plant systems

Plants provide a native-like cellular environment for the biosynthesis of complex plant-derived natural products, offering advantages such as correct enzyme folding, subcellular compartmentalization, and photosynthetically driven precursor supply. Among plant hosts, tobacco has emerged as a leading model for engineering the Taxol biosynthetic pathway. Several studies have reported successful production of taxadiene through both transient and stable expression strategies. For instance, Fu et al. [60] expressed a chloroplast-targeted TXS in transgenic tobacco, achieving yields of up to 87.8 $\mu g \cdot g^{-1}$ dry weight. Further enhancement was accomplished through overexpression of 1-deoxy-D-xylulose-5-phosphate synthase (DXS), a rate-limiting enzyme of the methylerythritol phosphate (MEP) pathway, resulting in up to eight-fold increases in taxadiene levels [61]. More recently, rerouting GGPP and taxadiene biosynthesis from the chloroplast to the cytosol led to a ten-fold improvement ($1.25\ mg \cdot g^{-1}$) over chloroplast-localized production, highlighting the importance of compartment-specific pathway optimization (Table 1) [62–64]. Jiang et al. [19] and McClune et al. [23] reconstituted the biosynthetic pathway for baccatin III in *N. benthamiana* through transient expression of multiple taxoid biosynthetic genes, although in the latter case, endogenous tobacco enzymes were likely also involved in completing the pathway. In a complementary approach, Liang et al. [24] reconstructed the downstream pathway in tobacco, enabling the biosynthesis of Taxol from the intermediate baccatin III. Beyond tobacco, alternative plant platforms such as tomato have also shown promise. In a notable example, TXS was expressed in a carotenoid-deficient yellow-fruited tomato line, enabling production of up to $160\ mg \cdot kg^{-1}$ of highly pure taxadiene in freeze-dried fruit tissue [65], demonstrating the feasibility of using edible crops for pathway engineering and downstream applications. Despite these advances, current plant-based systems face limitations, particularly with respect to transient expression systems and relatively modest product titers. Moving toward commercial viability will require overcoming metabolic bottlenecks, achieving stable gene expression, and increasing flux through the pathway in fully transformed lines.

3.3. Microbial systems

Microbial hosts offer a scalable, controllable, and potentially cost-effective platform for the production of Taxol and its intermediates [66]. These systems are particularly attractive due to their amenability to genetic engineering and rapid growth. Among microbial hosts, *E. coli* has been extensively employed owing to its well-characterized genetics and ease of manipulation. The first successful heterologous production of taxadiene was reported in *E. coli* in 2001, with titers reaching $1.3\ mg \cdot L^{-1}$ [67]. A major breakthrough was achieved by Ajikumar et al. [63], who employed a modular metabolic engineering approach that increased taxadiene production to nearly $1\ g \cdot L^{-1}$—the highest yield reported at the time. However, downstream steps in Taxol biosynthesis require multiple cytochrome P450-mediated oxidation reactions, which pose significant challenges in *E. coli* due to its lack of an endomembrane system. In contrast, the eukaryotic host yeast represents a promising alternative for downstream pathway expression due to its eukaryotic nature, robust membrane systems, and efficient post-translational processing machinery [68,69]. These features make it especially suitable for expressing membrane-bound and redox-active enzymes, including P450s. Engineered yeast strains have successfully produced oxygenated taxane intermediates such as taxadiene-5α-ol and its acetate derivative [21]. Nevertheless, despite its advantages in enzyme compatibility, yeast suffers from relatively low taxadiene titers compared to *E. coli,* limiting overall system productivity. In parallel, cyanobacteria have gained attention as a sustainable production chassis due to their ability to harness sunlight and fix carbon dioxide, thereby reducing reliance on organic carbon sources and lowering overall production costs. Leveraging these advantages, Zhong et al. [27] engineered *Synechocystis* sp. PCC 6803 with modular pathways comprising both MEP pathway enzymes and Taxol upstream biosynthetic enzymes, enabling the photosynthetic production of taxadien-5α-ol at titers of $4.32\ mg \cdot L^{-1}$ [28].

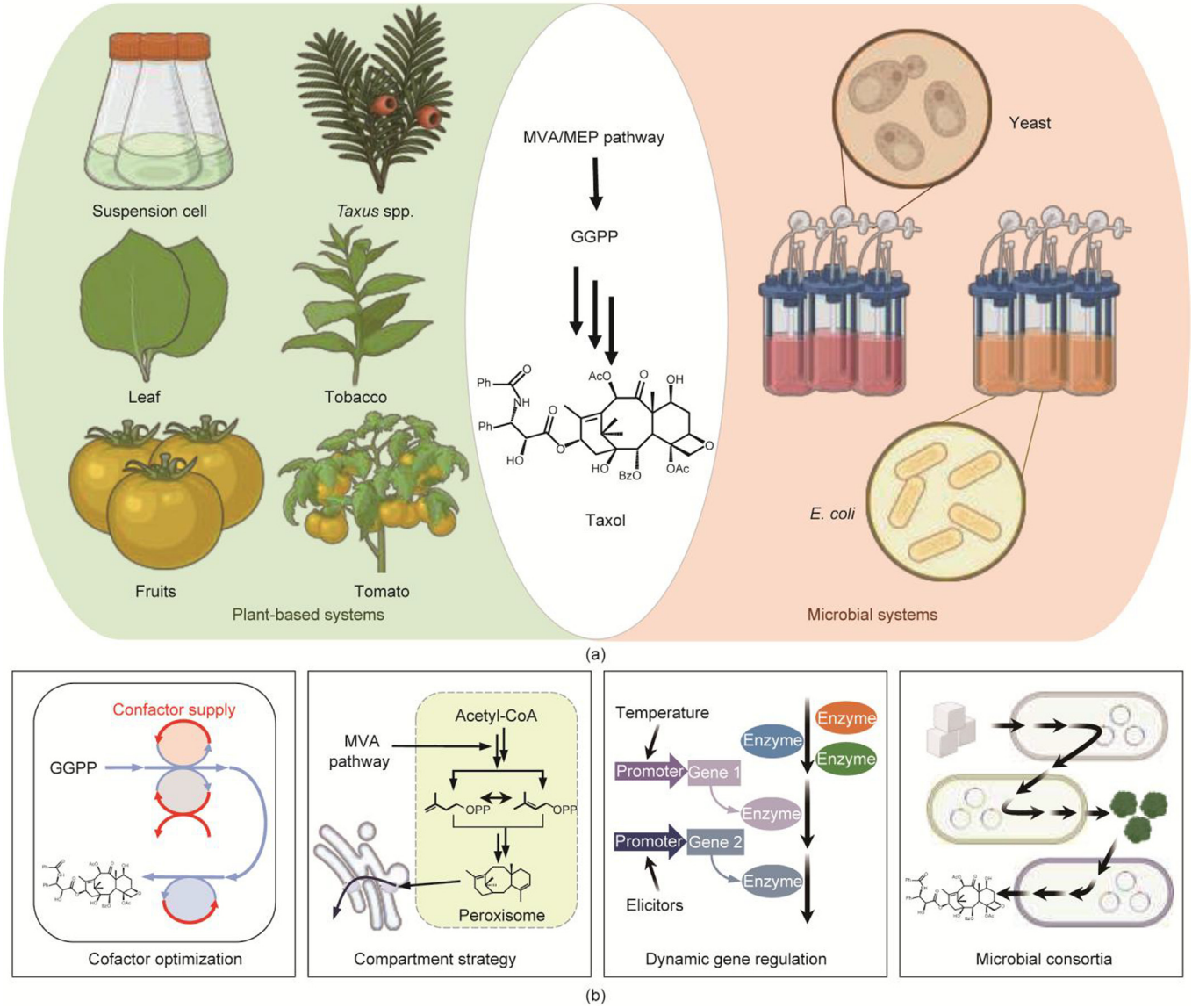


Fig. 4. Biological systems for Taxol production. (a) Overview of plant-derived and engineered microbial platforms employed for Taxol biosynthesis (b) Strategies in metabolic engineering to enhance Taxol production in heterologous hosts. MVA: mevalonate; MEP: methylerythritol phosphate.

Table 1
Maximum reported titer of Taxol intermediates in *N. benthamiana*, *E. coli*, and *S. cerevisiae* expression platforms.

Expression system	Taxa-4(5),11(12)-diene	Taxadien-5α-ol	Baccatin III
N. benthamiana	1.25 $mg \cdot g^{-1}$ [62]	1.3 $\mu g \cdot g^{-1}$	154.87 $ng \cdot g^{-1}$
E. coli	1 $g \cdot L^{-1}$ [63]	58 $mg \cdot L^{-1}$ [63]	—
S. cerevisiae	215 $mg \cdot L^{-1}$ [64]	43.65 $mg \cdot L^{-1}$ [64]	—

4. Conclusion and future perspectives

Advancements in elucidating the Taxol biosynthetic pathway have enabled its partial reconstruction in both plant and microbial hosts, marking a major step toward the sustainable production of this clinically important anticancer compound. Plant-based systems such as tobacco provide a native-like environment for the expression of plant-derived enzymes and have supported the successful integration of multiple pathway genes. As a result, they serve as valuable platforms for functional pathway characterization and discovery. In contrast, microbial systems offer distinct advantages for industrial-scale production. Yet, reconstituting the full Taxol biosynthetic pathway in a single microbial host remains highly challenging. The complexity and length of the pathway, involving more than 20 enzymatic steps, pose significant metabolic and engineering burdens. In particular, achieving efficient expression of key enzymes, such as cytochrome P450s and β-phenylalanyl-CoA ligases, within a single microbial chassis continues to be a technical hurdle.

Looking ahead, the rapidly evolving metabolic engineering toolbox offers promising strategies to address the persistent challenges associated with Taxol biosynthesis. As these approaches continue to advance, they hold the potential to unlock sustainable microbial production of Taxol. At the intracellular level, several advanced strategies have proven essential for enhancing the productivity of complex biosynthetic pathways such as that of Taxol. Cofactor optimization is a particularly effective approach, especially given the redox-intensive nature of Taxol biosynthesis. In yeast, engineering the supply and recycling of cofactors such as nicotinamide

adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) has led to substantial increases in the production of phenolic acids, which are key precursors for many plant-derived pharmaceuticals [70]. Applying similar cofactor engineering principles to Taxol biosynthesis could improve the efficiency of oxygenation steps, many of which are catalyzed by redox-dependent enzymes such as cytochrome P450s and OGDs.

Subcellular compartmentalization offers another powerful layer of control by creating tailored physicochemical environments for different pathway segments. In *S. cerevisiae*, directing enzymes to specific organelles, such as peroxisomes [71] or mitochondria [72], has significantly enhanced the biosynthesis of various terpenoids, including taxadiene [22,73,74]. This spatial organization mimics the metabolic architecture of plant cells, protects host metabolism from toxic intermediates, and minimizes unintended pathway crosstalk. These benefits are highly relevant to Taxol biosynthesis, including several oxygenation and acylation reactions that may be more efficiently catalyzed in specialized intracellular environments.

In addition to static pathway design, dynamic gene regulation systems have become essential tools for adapting gene expression in real time to the host's metabolic state. These systems, ranging from inducible and temperature-responsive promoters to feedback circuits and metabolite-responsive biosensors, enable fine-tuned, condition-dependent control of pathway genes [75–78]. This adaptive regulation helps maintain metabolic homeostasis, prevent the accumulation of toxic intermediates, and balance resource allocation between growth and production. In *S. cerevisiae*, for instance, feedback-regulated and metabolite-responsive promoters have been successfully deployed in the mevalonate pathway, enabling real-time modulation of precursor flux in isoprenoid biosynthesis [78], a pathway that shares early intermediates with Taxol. These regulatory strategies can be readily adapted to further refine Taxol pathway performance in microbial hosts.

At the pathway architecture level, modular engineering allows for the rational assembly and tuning of pathway components. This has enabled the successful reconstitution of complex plant alkaloid biosynthetic pathways, such as tropane [79] and vinblastine [16], in yeast, using a stepwise approach in which individual modules were optimized before integration.

Beyond single-species platforms, synthetic microbial consortia and co-culture strategies offer a compelling alternative to traditional monocultures. In monoculture systems, the entire biosynthetic burden, including precursor supply, cofactor regeneration, and expression of complex enzymes, is placed on a single host, often resulting in metabolic stress, reduced growth, and suboptimal yields. In contrast, multi-species consortia allow for functional division of labor, where different pathway modules are distributed across specialized strains or chassis. This modularity reduces cellular stress, improves pathway stability, and enhances overall scalability. Moreover, combining distinct expression systems, such as prokaryotic and eukaryotic hosts, enables the coordinated expression of enzymes with diverse requirements, such as cytochrome P450s and glycosyltransferases. For instance, Zhou et al. [80] divided the biosynthetic pathway of an acetylated diol taxane precursor between *E. coli* and yeast, establishing a stable co-culture through a mutualistic exchange of intermediates. This consortium achieved a titer of 33 $mg \cdot L^{-1}$ of oxygenated taxanes [80]. Similarly, Brooks et al. [81] developed a modular tripartite *E. coli* system for phenylpropanoid production, enabling flexible reconfiguration, stable expression, and reduced enzyme promiscuity. In a complementary approach, Chen et al. [82] engineered a mutualistic *S. cerevisiae* consortium using auxotrophic cross-feeding to divide lignan biosynthesis across two strains, improving pathway fidelity and glycosylation efficiency. These strategies highlight the potential of distributed biosynthesis for complex natural products, in this case, Taxol. As a result, further development of microbial consortia systems, leveraging different microbial hosts, will be crucial for advancing Taxol production. Future applications will benefit from the integration of modular strain design, metabolic insulation, and scalable fermentation strategies.

In conclusion, while significant hurdles remain, the integration of synthetic biology, metabolic engineering is rapidly transforming the landscape of natural product biosynthesis. Continued interdisciplinary innovation will be key to realizing efficient, high-titer Taxol production, and may serve as an example for the heterologous synthesis of other complex plant-derived pharmaceuticals.

CRediT authorship contribution statement

Yuxun Zhu: Writing – original draft, Resources, Methodology, Investigation. **Feiyan Liang:** Writing – original draft, Resources, Methodology, Investigation, Funding acquisition. **Pengpei Chai:** Writing – original draft, Resources, Methodology, Investigation. **Sotirios C. Kampranis:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Yong Zhao:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Feiyan Liang, Sotirios C. Kampranis and Yong Zhao are co-inventors on two patent applications for the biotechnological production of Taxol (PCT/EP2024/088095 and EP24386083.0). The other authors declare that they have no conflict of interest or financial conflicts to disclose.

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