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Review

Paclitaxel: biosynthesis, production and future prospects

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Plants are capable of producing a wide variety of secondary metabolites which have a diverse range of functions that can be exploited for medicinal purposes; for example, paclitaxel is a major anti-cancer drug found in the bark of *Taxus* spp. There are however supply issues as the compound is only found at low concentrations (0.05%) within the plant. The complex paclitaxel biosynthetic pathway makes chemical synthesis non-commercially viable; therefore alternative biotechnological sources have been explored for production including heterologous expression systems and plant cell culture.

Contents

Introduction

Paclitaxel is a highly successful cancer drug with a total market value of over $1 billion per year [1]. It was originally isolated from the bark of *Taxus brevifolia* in 1962 and is currently approved for the treatment of breast, lung and non-small cell cancers; however, its uses are predicted to expand [2]. The mode of action of paclitaxel was novel when it was first discovered in 1979 and involves the arrest of the cell cycle in the G_2/M phase by stabilizing microtubule formation [3]. The cost of paclitaxel is high at $600,000 per kilogram [4] because the drug is present at extremely low concentrations in the bark, for example 0.001–0.05% in *T. brevifolia* [1]. Research has been conducted to try and find alternative sources to natural harvesting of the compound including; (1) chemical synthesis, (2) semi-synthesis, (3) heterologous expression systems and (4) plant cell culture. However the structural complexity and an incomplete biosynthetic pathway have caused challenges.

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Paclitaxel biosynthetic pathway

Paclitaxel is a complex tetracyclic diterpene. The biosynthetic pathway is believed to contain nineteen steps; however, there are certain transformations which have yet to be fully characterized (Fig. 1).

The biosynthesis of paclitaxel involves the condensation of the three isoprenyl diphosphate (IPP) units with dimethylallyl diphosphate (DMAPP). Plants are unique in producing IPP and DMAPP by both the mevalonic pathway (MVA) in the cytosol or via the methylerythritol phosphate (MEP) pathway in the plastids (Fig. 1) [5]. The origin of the precursors involved in paclitaxel biosynthesis remains unclear [6]. Research with *Taxus baccata* shows that paclitaxel production can be reduced by inhibitors of both the cytosolic and plastid pathways [1].

The first committing step in the pathway is the cyclization of geranyl geranyl diphoshate (GGPP) to taxadiene, catalyzed by taxadiene synthethase (TASY). The tricyclic structure produced subsequently undergoes numerous regio- and stereospecific

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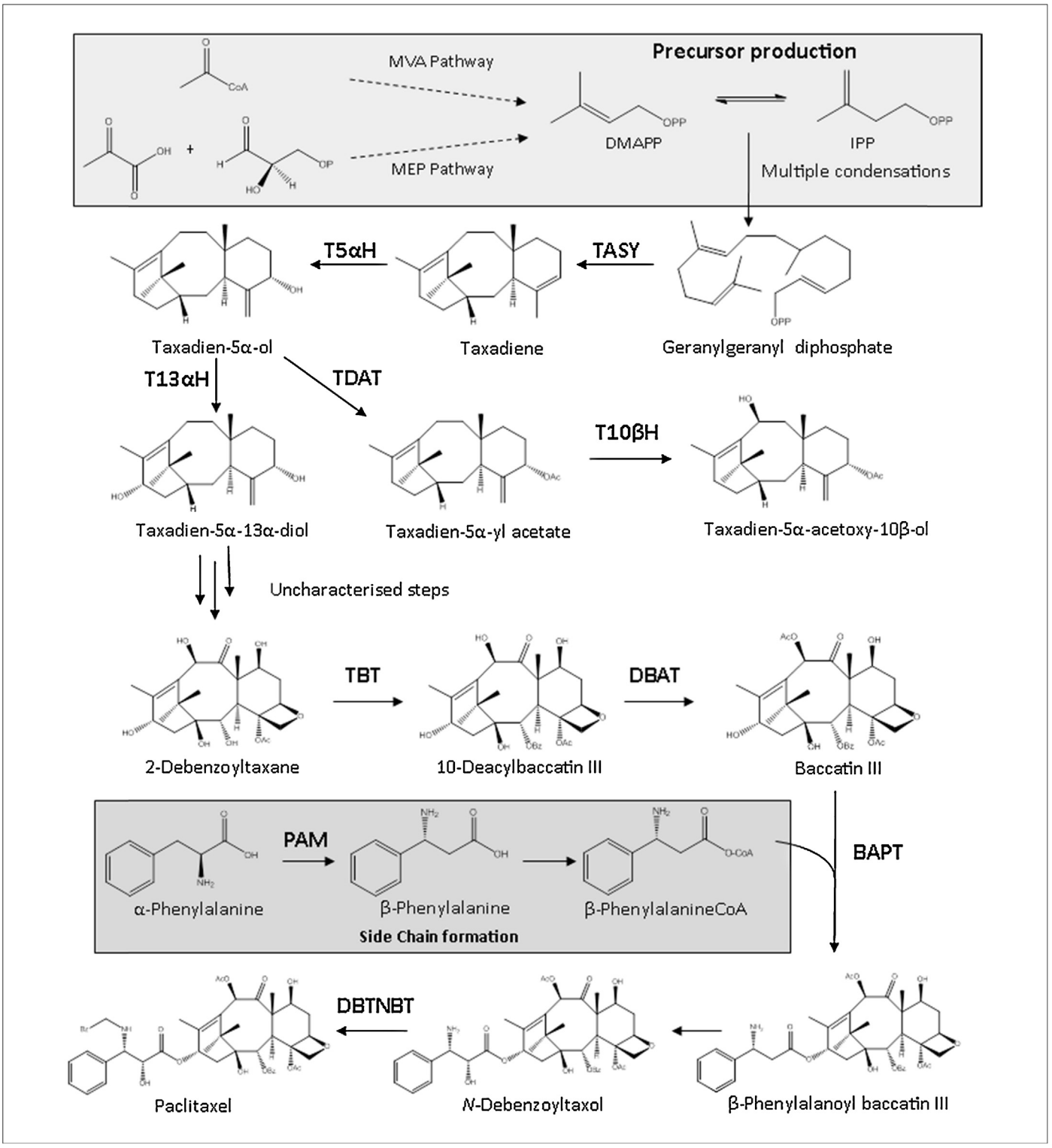

FIGURE 1

The paclitaxel biosynthetic pathway.
Adapted from Malik et al. [1] and Roberts [5].

oxygenations and acylations mediated by a variety of cytochrome P450 (cP450) oxygenases and acyltransferases. Taxadiene is then hydroxylated at the 5-position to produce taxadien-5α-ol by the cP450 taxadiene-5α-hydroxylase (T5αH). This intermediate can then undergo two possible transformations to either produce: taxadien-5α-yl-acetate by the enzyme taxadiene-5α-ol-*O*-acetyl transferase (TDAT) or taxadien-5α-13α-diol by the cP450 taxadiene-13α-hydroxylase (T13αH). The taxadien-5α-yl-acetate intermediate is then hydroxylated at the 10-position by cP450-dependent monooxygenase taxoid-10β-hydroxylase (T10βH) (Fig. 1).

The intermediate then undergoes four further hydroxylations at the C1, C2, C4 and C7 positions, an oxidation at C9 and an epoxidation between C4 and C5 [1]. The enzymes for these steps

have not been fully characterized but the hydroxylations are mediated by cP450's. A key unknown enzyme is the one involved in the production of the oxetane ring, which is essential for its activity [7]. There are several hypothetical mechanisms proposed, with the preferred candidate involving the epoxidation of the C4-C20 double bond of the 5α-acetate intermediate, followed by a rearrangement reaction where the α-acetoxy group migrates from C-5 to C-4 which is accompanied by the oxirane-to-oxetane ring expansion [8].

Four ester functionalities are also attached, which are; benzonate at C2, acetate at C4 and C10 and *N*-benzoyl-3-phenylisoserinoyl at C13. The order in which these occur is still unknown and elucidating the sequence is more problematic than for oxygenations because acylations lead to a large number of side products, many of which are known to have multiple acylation sites [9].

These transformations produce the hypothetical precursor 2-debenzoyltaxane for the enzyme taxane 2α-*O*-benzoyltransferse (DBT) to produce 10-deacetylbaccatin III, which is subsequently acetylated at the C10 position by 10-deacetylbaccatin III-10-*O*-acetyltransferse (DBAT). The C13 side chain is formed from α-phenylalanine, which is then converted into β-phenylalanine by phenylalanine aminomutase (PAM) and subsequently esterified by an unknown enzyme to produce β-phenylalanineCoA. This is then attached to the taxane core by the enzyme baccatin III-13-*O*-phenylpropanoyl transferase (BAPT) to produce 3′-*N*-debenzoyl-2′-deoxytaxol. This intermediate is hydroxylated at the C2 positon by an unknown cP450 and is converted in the final compound paclitaxel by the enzyme 3′-*N*-debenzoyl-2′-deoxytaxol-*N*-benzoyl transferase (DBTNBT) (Fig. 1).

Taxus spp. are capable of producing a variety of different taxanes [10]. The production of taxanes is most probable to occur via a branched rather than linear pathway (suggested in Fig. 1), which generates this wide variety of different compounds. The proposal is supported by the identification of enzymes that are not involved in transformations leading to paclitaxel, for example, taxoid 14β-hydroxylase [9].

Perspectives for production

Paclitaxel was originally isolated from the bark of *Taxus* spp.; however natural harvest is not a viable option as levels of the compound are extremely low, yew is a slow growing species and the extraction is a destructive process. For one treatment 2.5–3 g of paclitaxel are needed, which requires eight mature yew trees [1].

A total synthesis for Taxol was first published in 1994 by two different labs, Holton et al. and Nicolaou et al. proposing different routes [11–13]. Even through subsequent schemes have been published, none of them are commercially viable because they are complex multi-step pathways, which produce low yields and numerous toxic side products.

A semi-synthetic process is currently employed commercially by Bristol Myers Squibb, which involves the extraction of paclitaxel intermediates baccatin III or 10-deacetylbaccatin III from the needles of *Taxus* spp. This process however has several disadvantages including; (1) the production is dependent on epigenetic and environmental factors, (2) *Taxus* is a slow growing species and (3) the intermediates require purification which is expensive.

Some steps of the paclitaxel biosynthetic pathway have been transferred into heterologous expression systems, including *Escherichia coli*, *Saccharomyces cerevisiae* and plants. The overexpression of IPP isomerase, GGPP synthase and TASY in *E. coli* led to production levels of 1.3 mg of taxadiene per liter of cell culture [14]. However further development of the pathway is limited due to the difficulties of expressing cP450's in microbial systems. cP450's often lose their functionality due to incorrect folding, translation and insertion into the cell membrane. Further, problems with cofactor availability together with the absence of the specific cP450 reductases required to enzymatically recycle each cP450 represent significant obstacles [15]. Five sequential paclitaxel biosynthetic genes were expressed in *S. cerevisiae* with the intention of producing taxadien-5α-acetoxy-10β-ol; however, only high (mg/L) levels of taxadiene were observed [16]. The pathway was restricted at the first cP450 hydroxylation, which may require the co-expression with its cognate cP450 reductase for efficient function [17].

Several plant hosts have also been exploited to produce paclitaxel intermediates. GGPP synthase and TASY were overexpressed in *Arabidopsis thaliana* leading to the production of 600 ng of taxadiene per gram of dry weight. However the introduction of these exogenous genes leads to growth retardation due to the accumulation of taxadiene [18]. Tomato plants over expressing TASY and lacking the ability to utilize GGPP for carotenoid synthesis produced 160 mg of highly pure taxadiene from 1 kg of freeze dried fruit [19]. The overexpression of TASY in *Physcomitrella patens* produced 0.05% fresh weight of taxadiene with no growth inhibition [20]. Collectively, these attempts to synthesise paclitaxel in plant and microbial production hosts highlight that the paclitaxel biosynthetic pathway is highly complex and our understanding is incomplete.

Plant cell culture (PCC) is seen as a viable alternative to the previously mentioned production routes. Several advantages of PCC include: (i) production is independent of geographical and seasonal variations; (ii) the technique provides a continuous and uniform quality Taxol; and (iii) it is a renewable and environmentally friendly resource [4]. The first patent for PCC using *T. brevifolia* was filed in 1991 and produced 1–3 mg/L [21]. Currently two companies – Phyton Biotech USA and Samyang Genex, South Korea – produce paclitaxel on an industrial scale.

Issues associated with PCC include genetic instability, heterogeneous culture, low growth rates compared to bacterial cultures, variable yields, susceptibility to shear stresses in bioreactors and aggregation [22]. Research has been conducted in several areas to try and alleviate some of these issues. Elicitors such as methyljasmonate are capable of increasing paclitaxel in a variety of *Taxus* spp. to levels greater than 20 mg/L [23]. Traditional PCC is composed of dedifferentiated cells (DDC); however, work by Lee et al. initiated cultures using undifferentiated cambial meristematic cells (CMCs), which are multipotent with stem-cell-like properties. These cells have lower growth variability, smaller aggregates and were less sensitive to shear stresses. CMCs generated from *Taxus cuspidata*, produce higher levels of paclitaxel at 102 mg/kg fresh cell weight compared to only 23 mg/kg and 39 mg/kg for DDC derived from needles and embryos respectively [24]. Utilizing CMCs as a chassis for subsequently metabolic engineering strategies may further increase paclitaxel biosynthesis in these cells.

Conclusion

Collectively, the emerging data suggest that semi-synthetic procedures will continue to be required together with an increasing role for plant cell culture; especially as improved cell lines and the possibility of metabolic engineering comes into view. Efforts to fully dissect the biochemistry of this pathway remain an important goal. Finally, while a significant progress has been made in expressing a few enzymes integral to paclitaxel biosynthesis in microbial production hosts, it is clear that paclitaxel synthesis in such systems is a long-term goal.

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