Plant cytochromes P450: tools for pharmacology, plant protection and phytoremediation

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Cytochromes P450 catalyse extremely diverse and often complex regiospecific and/or stereospecific reactions in the biosynthesis or catabolism of plant bioactive molecules. Engineered P450 expression is needed for low-cost production of antineoplastic drugs such as taxol or indole alkaloids and offers the possibility to increase the content of nutraceuticals such as phytoestrogens and antioxidants in plants. Natural products may serve important functions in plant defence and metabolic engineering of P450s is a prime target to improve plant defence against insects and pathogens. Herbicides, pollutants and other xenobiotics are metabolised by some plant P450 enzymes. These P450s are tools to modify herbicide tolerance, as selectable markers and for bioremediation.

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Abbreviations
RT–PCR reverse transcription polymerase chain reaction

Introduction

Cytochromes P450 form the largest family of plant proteins. The databases currently list 1052 P450 sequences obtained from a small subset of all known plant species (http://drnelson.utmem.edu/CytochromeP450.html; http://www.biobase.dk/P450/; http://*Arabidopsis*-p450.biotec.uiuc.edu/). Evolution of this huge superfamily of enzymes is obviously related to the complex and versatile chemistry developed by higher plants to synthesise a large number of natural products that enable plants to communicate with other plants, attract pollinators and deter pathogens and herbivores. P450 heme proteins catalyse most of the oxidation steps in plant secondary metabolism resulting not only in simple hydroxylations or epoxidations, but also in more complex reactions ranging from dealkylations to isomerisations and aryl migrations, decarboxylations, dehydrations, ring formation, ring extension, phenol coupling and carbon–carbon bond cleavages [1,2]. These diverse reactions are needed for the synthesis of the fantastic diversity of plant natural products. A large proportion of the most complex regiospecific and stereospecific steps in the biosynthesis of bioactive compounds is catalysed by P450 enzymes. Such oxidation reactions are usually slow compared with other steps in the same pathway and difficult to perform by synthetic chemistry. The full catalytic potential of plant P450s is still far from understood.

Natural products are an obvious target for the design of plants with improved nutritional value and for creating herbivore and pest-resistant plants. Transgenic plants in which the metabolic engineering of cytochromes P450 has resulted in improved insect resistance have now been produced. The huge number of duplications of P450 genes in higher plants has allowed recruitment of new functions (e.g. the evolution of efficient catalysts for detoxification of exogenous molecules). Although knowledge remains scarce on this aspect of plant P450 function, the first examples of P450 genes encoding xenobiotic metabolising enzymes show that they can be used for engineering herbicide or pesticide tolerance in crops, for bioremediation or as selectable markers of plant transformation. In addition, natural products are important to humans as flavours, condiments, aroma compounds, nutraceuticals and as drugs for the treatment of a large number of diseases (e.g. neoplasms). About 25% of contemporary medicines are derived from plants and secondary metabolites still serve as leads for synthetic drugs.

The purpose of this review is to provide a summary of the most recent advances in our knowledge of cytochromes P450 and their role in the formation and transformation of natural products and xenobiotics. The new and exciting possibilities for biotechnological applications of P450s are discussed.

Bioproduction of antimitotic drugs

Some very complex natural products with important and potent biological activity cannot be produced at reasonable costs by chemical synthesis. Their availability relies on natural sources of the final compound or of some advanced

precursor that is more easily converted to the desired compound using synthetic chemistry. Enhancement of the biological production of these compounds is necessary to extend the availability of such drugs to the general public in the western world and to developing countries where exorbitant prices would otherwise prevent their use. This is highly important for the two very useful classes of chemotherapeutic agents discussed below.

Taxoids

Taxoids provide one of the best illustrations of the prominent role of P450s in a drug biosynthetic pathway [3–5]. The diterpenoid taxol (paclitaxel) is a potent antimitotic agent with excellent activity against different types of carcinomas, melanomas and sarcomas. It is, however, in limited supply from its original source, the bark of Pacific yew (*Taxus brevifolia*). This prompted development of alternative means of production such as a hemisynthetic approach based on more readily available and renewable natural sources (i.e. the needles or other tissues of different yew species). Increased demands for taxol and its high cost have recently accelerated research towards elucidation of its biosynthetic pathway. The first committed step in the biosynthesis of taxol is the cyclisation of geranylgeranyl diphosphate to taxa-4(5),11(12)-diene. This is followed by extensive oxidative modification and acylation of the taxane skeleton for elaboration of the sidechains (Figure 1a). The first experimental evidence for P450-mediated oxygenation at the eight positions of the taxane ring were obtained by nuclear magnetic resonance (NMR) analyses of the metabolites formed after administration of $^{18}O_2$ to a *Taxus* cell culture [6]. The results were substantiated [3,7] by incubation of different precursors with microsomes prepared from woody tissues of *Taxus* [8]. Unravelling the sequential order of functionalisation was guided by the relative abundance and substitution patterns of the large range of naturally occurring taxoids with known structure. First, hydroxylation was predicted to occur at C5 with subsequent allylic migration of the double bond. The prediction was confirmed by the demonstrated conversion of taxa-4(5),11(12)-diene into taxa-4(20),11(12)-dien-5-ol using microsomes from stems and cell suspension cultures [9]. Precursor feeding experiments with stem microsomes also indicated that this oxygenation was rate limiting relative to subsequent transformations.

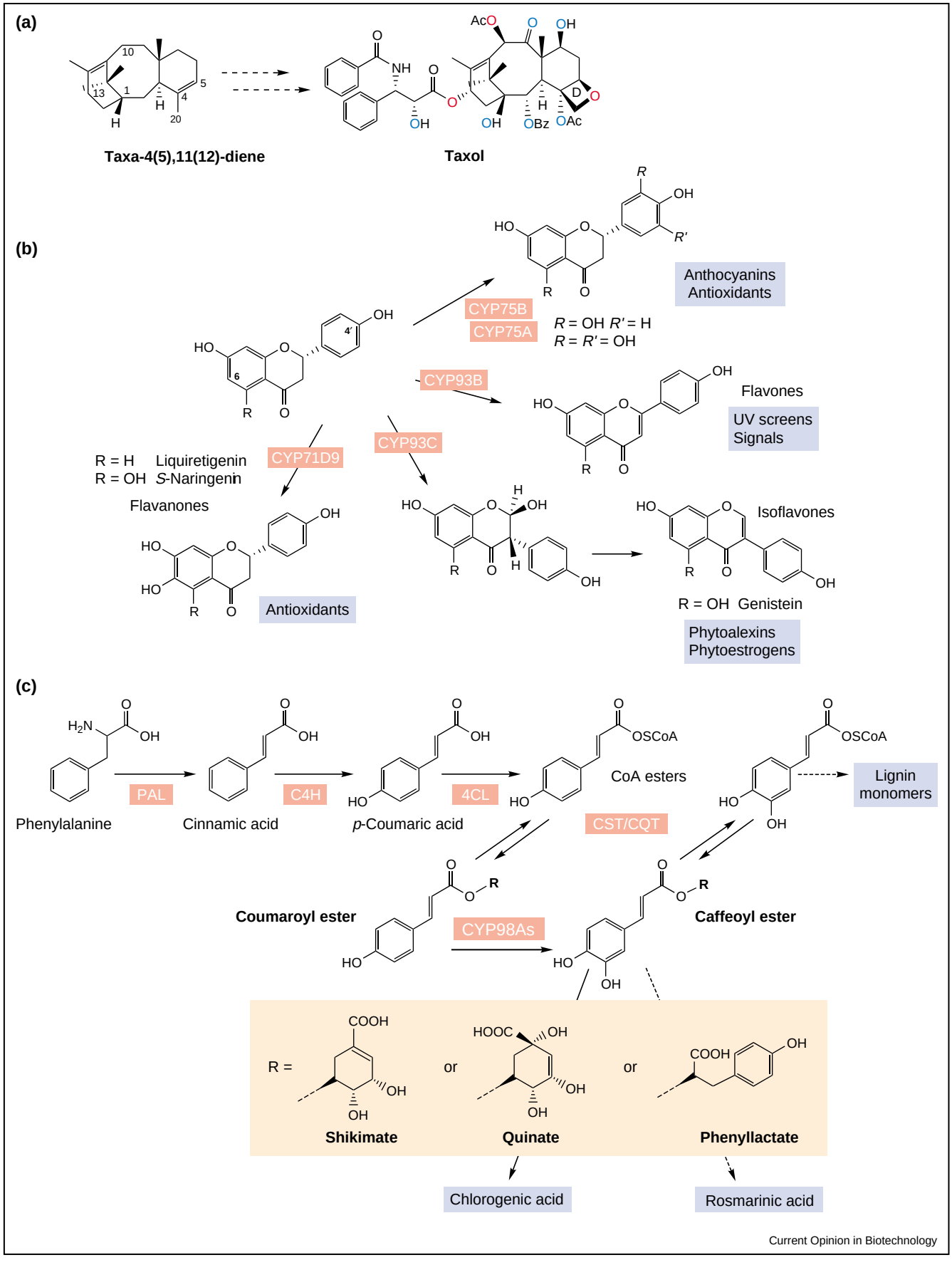
Because cloning of *Taxus* P450 genes by classical approaches was not practicable, an alternative strategy was designed based on the use of cell suspension cultures elicited to achieve maximal taxol production [10]. Using an uninduced *Taxus* culture as a control source for isolation of mRNA, transcriptionally active P450 genes were identified by reverse transcription-polymerase chain reaction (RT–PCR) differential display [11••]. This led to characterisation of taxane 10β-hydroxylase, the P450 catalysing the second oxygenation step in taxol biosynthesis, and the isolation of 12 other related P450 genes likely to catalyse other oxygenation steps in the taxol pathway. These genes show phylogenetic relationships with genes involved in the synthesis of other large terpenoids (e.g. gibberellins and steroids). Expression in yeast or insect cells already confirmed metabolism of taxoids by several of these P450s, the first being a taxane 13α-hydroxylase [12••]. It seems possible that all of the genes controlling oxygenation steps in taxol biosynthesis will soon be described. The characterisation of genes encoding enzymes responsible for acylations at the different positions of the taxane ring is proceeding at a similar pace [4] and the knowledge required for extensive engineering of the taxol biosynthetic pathway in recombinant microorganisms, cultured cells or fast growing *Taxus* species should soon be available. Data already available from analyses with cultured cells, cell-free extracts or recombinant enzymes suggest the existence of multiple pathways or dead-end branches that offers good possibilities not only for metabolic engineering towards increased production of taxol, but also for the production of new generations of more active taxoids [5,7,10].

Terpenoid indole alkaloids

Terpenoid indole alkaloids form a large group of structurally diverse molecules with interesting pharmaceutical activities such as the antineoplastic agents vincristine and vinblastine, the antihypertensives reserpine and ajmalicine, and the anti-arrhytmic ajmaline. Such compounds are found in a limited number of plant species including Apocynaceae, Loganiaceae, Rubiaceae and Nyssaceae and are difficult to isolate in sufficient amounts from the plant source. The high commercial prices of terpenoid indole alkaloids has stimulated research into the elucidation of their biosynthetic pathways. This work recently

(Figure 1 Legend) Cytochromes P450 in the synthesis of drugs and nutraceuticals. **(a)** Oxygen atoms inserted in the molecule of taxol by the action of cytochromes P450 are indicated in red. Additional oxygen atoms likely to be inserted by P450s are indicated in blue. A P450 may also be involved in the formation of the oxetane D-ring. **(b)** At least five different P450s are involved in the modification of the basic flavonoid structure found in all plant species. The relative expression of these P450s controls accumulation of the end-chain metabolites to form pigments, co-pigments, UV screens, signals for pollinators and symbionts, antioxidants, phytoalexins and phytoestrogens. A prerequisite for accumulation of the end-chain metabolites is the activation of the upstream flavonoid pathway. CYP75A, flavonoid 3′,5′-hydroxylase; CYP75B, flavonoid 3′-hydroxylase; CYP93B, flavone synthase II; CYP93C, 2-hydroxyisoflavone synthase; CYP71D9, flavonoid 6-hydroxylase. **(c)** 3-Hydroxylation of *p*-coumaric acid to form caffeic acid proceeds via shikimate, quinate or other esters and is catalysed by CYP98A enzymes. CYP98 enzymes are thus involved in the synthesis of chlorogenic and rosmarinic acids. PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-(hydroxy)cinnamoyl CoA ligase; CST, hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase; CQT, hydroxycinnamoyl-CoA:*D*-quinate hydroxycinnamoyl transferase.

Figure 1


(a)
Taxa-4(5),11(12)-diene
Taxol
(b)
CYP75B
CYP75A
R = OH R′ = H
R = R′ = OH
Anthocyanins
Antioxidants
CYP93B
Flavones
UV screens
Signals
CYP93C
CYP71D9
R = H Liquiretigenin
R = OH S-Naringenin
Flavanones
Antioxidants
Isoflavones
R = OH Genistein
Phytoalexins
Phytoestrogens
(c)
Phenylalanine
PAL
Cinnamic acid
C4H
p-Coumaric acid
4CL
CoA esters
CST/CQT
Lignin
monomers
Coumaroyl ester
CYP98As
Caffeoyl ester
R =
Shikimate
or
Quinate
or
Phenyllactate
Chlorogenic acid
Rosmarinic acid
Current Opinion in Biotechnology

led to the demonstration of P450 involvement in the 16-hydroxylation of the bisindole alkaloid precursor tabersonin in *Catharanthus roseus* [13] and in the conversion of loganin into secologanin and 7-hydroxylation of deoxyloganin in *Lonicera japonica* [14,15]. Biochemical characterisation of the enzymes was rapidly followed by isolation of several *C. roseus* P450 genes in the pathway and confirmation of their function in recombinant systems: CYP71D12 as a tabersonine 16-hydroxylase [16], CYP72A1 as a secologanin synthase [17••], and CYP76B6 as a geraniol/nerol 10-hydroxylase [18]. CYP72A1 is the first example of a P450 from a higher plant to catalyse a ring-opening reaction, probably via a radical mechanism. Precursor feeding experiments have suggested that conversion of loganin to secologanin is a potential rate-limiting step in indole alkaloid biosynthesis [19]. Characterisation of the genes encoding CYP76B6 and CYP71D12 is also important, because they catalyse the initial and rate-limiting steps for the synthesis of the terpenoid moiety secologanin and the strictly controlled production of vindoline, respectively [20]. Expression of both genes was found to be extremely responsive to elicitation or growth conditions [16,18,21]. They are thus likely to constitute limiting factors for alkaloid production in cell suspension cultures. The isolation of genes encoding key enzymes provides an important tool to investigate how pathways are regulated by light, jasmonate, nutritional downshift, barbiturates or cellular differentiation [16,18,21,22••,23••]. Knowledge of key genes can also be used to enhance alkaloid production in plant cell cultures [23••], for the design of recombinant microorganisms as alternative sources for alkaloid production [24] and for metabolic engineering of alkaloid producing plants. The latter might include increasing the expression of enzymes that catalyse rate-limiting steps, suppressing undesirable side routes, optimising co-localisation of enzymes in desired tissues and in cellular compartments to enhance the possibility for metabolon formation, and increasing flux through the pathway using transcriptional regulators.

In addition to the remaining unidentified steps in the biogenesis of indole alkaloids, P450s catalyse a large number of reactions in the biosynthesis of other alkaloids; for example, P450s have a key role in the formation of the parent ring systems that determine the class of alkaloids found in specific plant taxa. These P450s, their characteristics and biotechnological potential have recently been reviewed [25].

Crops with enhanced nutritional value: nutraceuticals and antioxidants

More straightforward opportunities to enhance the production of health-beneficial compounds are found in the phenylpropanoid or glucosinolate pathways in which expression of a single gene or a few additional genes is sufficient to promote the production of desired natural products.

In the biosynthesis of flavonoids, common precursors are used for the formation of compounds with different ring systems and substitution patterns of the rings. The ring and substitution patterns dictate the type of bioactivity obtained (Figure 1b). Several genes encoding P450s that catalyse branch-point reactions in flavonoid biosynthesis have recently been characterised. *CYP93C* encodes the 2-hydroxyisoflavone synthase (also simplified as isoflavone synthase), which catalyses B-ring aryl migration from C2 to C3 of naringenin or liquiretigenin to produce isoflavonoids [26,27,28•]. CYP93C and isoflavonoids have a restricted distribution in the plant kingdom and are found almost exclusively in legumes, in particular soybean. The simplest isoflavonoid and the direct product of CYP93C is genistein. Genistein shares structural features with estrogens. Its high abundance in Asian diets has been implicated as a causal agent in the reduced incidence of cancer and cardiovascular diseases in Asia, with reduced post-menopausal symptoms as an additional health benefit [29•]. Such compounds have therefore been referred to as phytoestrogens and are considered prime candidates for engineering health-promoting crops. Preliminary attempts to use metabolic engineering to introduce genistein production into otherwise isoflavonoid-free dicotyledoneous and monocotyledoneous species have been reported. A lesson from these studies is that if the expression of CYP93C is eliminated as a rate-limiting factor through metabolic engineering, the availability of its substrate naringenin becomes a new rate-limiting factor [30••]. This limitation can be relieved in a tissue-specific manner or by the co-expression of flavonoid up-regulating transcription factors. A gene coding for another P450 (CYP71D9), which converts genistein and liquiretigenin to 6-hydroxylated compounds, has recently been isolated from elicited soybean cells [31,32]. Likewise, baicalein and wogonin are flavonoids hydroxylated in the 6 or 6,8 positions and found in the roots of the Chinese medicinal plant *Scutellaria baicalensis*. Such compounds have in recent years attracted considerable attention both as antioxidants and for their anti-inflammatory and anti-proliferative properties and constitute targets for crop improvement. Flavonoids are marketed as antioxidants with a positive health effect; however, they possess other biological activities (e.g. the ability to change the pharmacokinetics of drug uptake) that may not always be recognised or desired [33].

Other documented health-promoting phenolic compounds are caffeic acid and its derivatives. These molecules are potent antioxidants because, as in catechol, the aromatic ring contains free vicinal hydroxyl groups. The quinate and phenyllactate esters of caffeic acid (chlorogenic acid and rosmarinic acid, respectively) are described as antimicrobial, hepatoprotective, antiproliferative and antidepressive compounds. The 3-hydroxylation step required for the formation of these compounds has long

remained elusive. The enigma was recently resolved by the discovery that 3-hydroxylation to form caffeic acid has to proceed via shikimate, quinate or other esters of *p*-coumaric acid [34••,35,36•] (Figure 1c). P450s belonging to the CYP98A subfamily catalyse these reactions and their role was discovered by complementary approaches using functional genomics [34••] and forward genetics [37]. Expression of *CYP98A3* was shown to be required for synthesis of all caffeate derivatives, including lignin monomers in *Arabidopsis* [38••]. Together with the upstream transferases, which converts coumaroyl-CoA into shikimate or quinate coumaroyl esters [39], the availability of these genes offers the possibility to engineer the content of caffeoyl esters in fruits and crops (e.g. chlorogenic acid in coffee). They also provide a good starting point for metabolic engineering to enhance the content of flavours such as vanillin, eugenol and safrole or of floral scents.

Improved nutritional effects may also be obtained by removal of toxic constituents. Cassava contains very high levels of the cyanogenic glucosides linamarin and lotaustralin in all parts of the plant. The genes encoding enzymes that catalyse the first committed step in cyanogenic glucoside synthesis in cassava have recently been identified as *CYP79D1* and *CYP79D2* [40]. The availability of these genes together with a transformation system for cassava will make it possible within the next few years to obtain cassava plants that do not accumulate cyanogenic glucosides in their edible parts. As for cyanogenic glucosides, the initial step in glucosinolate synthesis is also catalysed by P450s belonging to the CYP79 family. Glucosinolates, also referred to as mustard oil glucosides, are derived from amino acids and contain a sulfate and thioglucose residue. Upon tissue damage, glucosinolates are brought into contact with myrosinases and hydrolysed into unstable aglycons. These are further converted into a range of biologically active compounds like isothiocyanates and nitriles that possess antimicrobial, cancer-preventing, goitrogenic or inflammatory activities depending on the nature of the glucosinolate precursor amino acid and the type of degradation product formed [40]. The CYP79s have a high substrate specificity that determines which cyanogenic glucoside or glucosinolate is produced in the particular plant species. Several *CYP79* genes have become available within the past few years that encode enzymes involved in the conversion of both aliphatic and aromatic amino acids into cyanogenic glucosides and glucosinolates [40,41•,42]. This offers the possibility to engineer crops with improved nutritional value and anticarcinogenic properties and to obtain plants with improved insect and pathogen resistance.

Improved plant defence

The primary role of natural products is to enable plants to communicate with the environment. They may act as toxins or biocides against competing plant species, pathogens, insects and other herbivores or as attractants of pollinators and parasitoids [43]. They may also act as stress signals activating plant defence.

Signalling molecules

One route towards improved plant protection is to engineer stress signalling pathways to increase or accelerate the formation of signalling molecules. Hydroperoxy fatty acids are generated by the coordinated action of lipases and lipoxygenases. These hydroperoxy fatty acids are substrates for the CYP74 family of P450s, which convert them to oxylipins — compounds with signalling functions in plant development and defence [44–46]. Some of these compounds exert a direct antifungal or antimicrobial activity [47]. Four CYP74 subfamilies control the entry of hydroperoxides into different pathways that may provide complementary, synergistic and overlapping contributions to plant defence. CYP74A enzymes are allene oxide synthases involved in the synthesis of jasmonates, as confirmed with *Arabidopsis thaliana* knockout mutants [48]. Overexpression of CYP74A is usually not sufficient to increase the basal levels of jasmonates, apparently because of limited substrate availability. Upon wounding of plants that overexpress CYP74A, higher and earlier jasmonate accumulation was indeed observed but no impact on plant defence has yet been described [48,49]. CYP74B enzymes are hydroperoxide lyases that produce the wound hormone traumatin and the volatile aldehydes hexanal and 3-hexenal that affect insect fecundity and serve as mild wound signals [50]. The impact of CYP74B on plant defence was investigated by an antisense approach. Potato plants with impaired aldehyde accumulation showed little variation in wound-responsive genes but exhibited significantly decreased resistance to aphid attack [51••]. While CYP74A triggers a jasmonate-mediated wound response to chewing insects, the CYP74B pathway appears to form a constitutive defence against sucking pests in plant foliage. The emerging picture is that CYP74A and CYP74B enzymes accumulate in photosynthetic tissues with a substrate preference for 13-hydroperoxides; they are usually located in the plastids [52]. Conversely, CYP74C and CYP74D enzymes exhibit a substrate preference for 9-hydroperoxides and are usually found constitutively expressed in roots or other non-photosynthetic tissues. CYP74C enzymes catalyse the same reactions as CYP74As and CYP74Bs, being either allene oxide synthases or hydroperoxide lyases, but with a marked substrate specificity for 9-hydroperoxides [53,54,55•]. Their exact role in defence and development is not well understood. CYP74D enzymes are divinylether synthases forming antimicrobial compounds such as colnelenic and colneleic acids in *Solanaceae*. Their pattern of expression suggests a role in built-in protection against pests in plant roots [56]. Accumulation of *CYP74D* transcripts and divinylethers is also induced in leaves in response to specific pathogens [57,58•].

Defence compounds

Another way to improve plant protection is to increase the accumulation of defence compounds. A typical example is the engineering of terpene exudates in tobacco glandular trichomes. In certain tobaccos, exudates may contribute as much as 16% to the leaf dry weight and the two main constituents are non-volatile cembranoid diterpene (cembra-2,7,11-triene-4,6-diol) epimers. Antisense or co-suppression of *CYP71D16*, a trichome-specific P450 gene, results in accumulation of cembratrieneol instead of cembratriene-diol [59••]. The modified exudate has a higher aphidicidal activity and *CYP71D16* downregulated plants show strikingly reduced aphid colonisation. The promoter of the *CYP71D16* gene has been isolated and constitutes a promising tool for metabolic engineering of trichome content to produce new useful metabolites and to enhance pest/disease resistance [60]. Two P450s recently characterised in *Solanaceae* hold similar promise to enhance plant resistance to fungal pathogens. CYP71D20 catalyses two successive hydroxylations in the formation of the sesquiterpene capsidiol, which is the primary antimicrobial compound in tobacco [61•]. The other P450 was recently identified as a marker of potato resistance to late blight and associated with a quantitative trait locus (QTL) [62].

Cyanogenic glucosides are β-glucosides of α-hydroxynitriles and are classified as phytoanticipins. Upon disruption of plant tissue containing cyanogenic glucosides, these are degraded by β-glucosidases and α-hydroxynitrilases resulting in the release of toxic hydrogen cyanide as well as glucose and an aldehyde or ketone. This binary system — comprising two sets of components that separately are chemically inert — provides plants with an immediate chemical defence response to herbivores and pathogens that cause tissue damage [63]. To specifically study the effect of cyanogenic glucosides on plant–insect interactions, the entire pathway for the synthesis of the tyrosine-derived cyanogenic glucoside dhurrin has recently been transfered into *A. thaliana* using the *Sorghum bicolor* genes *CYP79A1*, *CYP71E1* and *UGT85B* (Figure 2). *A. thaliana* plants ectopically expressing the three sorghum genes displayed no apparent physiological phenotype or impact on plant fitness, illustrating the capacity of plants to accommodate foreign biosynthetic pathways and to accumulate and handle novel natural products. *A. thaliana* belongs to the brassicaceae, which do not contain cyanogenic glucosides. In free-choice tests, the crucifer specialist flea beetle *Phyllotreta nemorum* was found to avoid the dhurrin-containing *A. thaliana* plants [64••]. The successful transfer of an entire pathway for a natural product into a new plant species and the obtained resistance to a specific insect clearly demonstrates the potential of metabolic engineering to generate crop plants with desired characteristics.

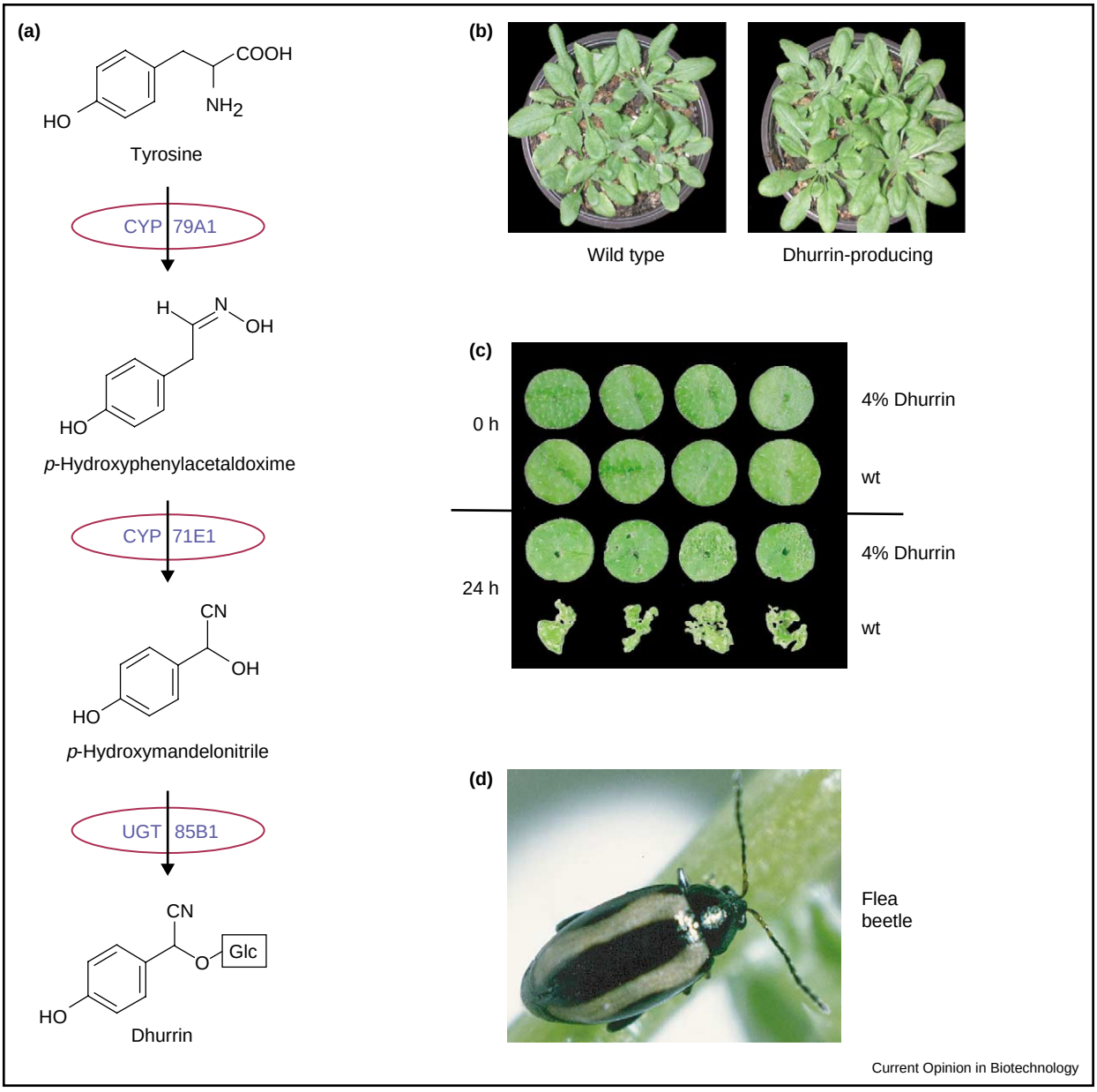
Engineered herbicide tolerance and phytoremediation

Concomittant with a demonstrated involvement in synthesis of natural products, plant P450s were shown to catalyse herbicide metabolism and to contribute to detoxification or activation of other agrochemicals in crop plants. These P450s are key determinants of herbicide tolerance and selectivity, and increases in their expression levels can be responsible for the appearance of herbicide cross-resistance in weeds [65]. P450-dependent metabolism of organic pollutants has also been reported or suggested [66–68]. Progress in the molecular characterisation of such P450s has been very slow due to limited research efforts and was probably further hampered because most herbicide-detoxifying P450s are expressed a low levels in plants.

The first two plant P450s shown to actively metabolise herbicides, CYP76B1 and CYP71A10, (Figure 3) were isolated from *Helianthus tuberosus* and soybean, respectively [69,70]. The first was isolated taking advantage of its inducibility by chemicals such as aminopyrine and Mn^{2+}. Both P450s metabolise phenylureas and no other herbicide classes, but showed different substrate regiospecificities and catalytic efficiencies, with CYP76B1 as the most active. Similarly, on the basis of chemical inducibility by the herbicide 2,4-D and herbicide safeners, CYP81B2 and CYP71A11 were isolated from tobacco and shown to metabolise chlortoluron, although at a very low rate [71]. In wheat, attempts to isolate herbicide-metabolising P450s using RT–PCR differential display [11••,31] were unsuccessful but resulted in isolation of a single herbicide safener inducible P450 (L Didierjean, M Morant, unpublished data). These efforts resulted in the design of new methods for targeted isolation of P450 genes from monocot crops and weeds [72,73•].

Because of the lack of relevant plant genes, the first attempts to engineer herbicide tolerance into plants were performed with P450 cDNAs isolated from bacteria and mammals. CYP105A1 isolated from *Streptomyces griseolus* catalyses the activation of a sulfonylurea proherbicide. This gene was targeted to the chloroplast to exploit the availability of electron supply from ferredoxin and further developed into a negative selection system for use in dicots [74] and monocots [75]. A small subset of 11 human P450 genes was shown to exhibit broad substrate specificity and to account for 90% of the metabolism of drugs and exogenous molecules. Such P450s, which also metabolise multiple herbicides [76], have been successfully used to introduce herbicide tolerance in model plants [77–79] and cell suspension cultures. For ethical and technical reasons such human transgenes are not likely to be transferred to field-grown crops; however, they are useful for the production of metabolites and for the prediction of potential P450-catalysed conversions of new agrochemical compounds as long as relevant genes are not available from higher plants.

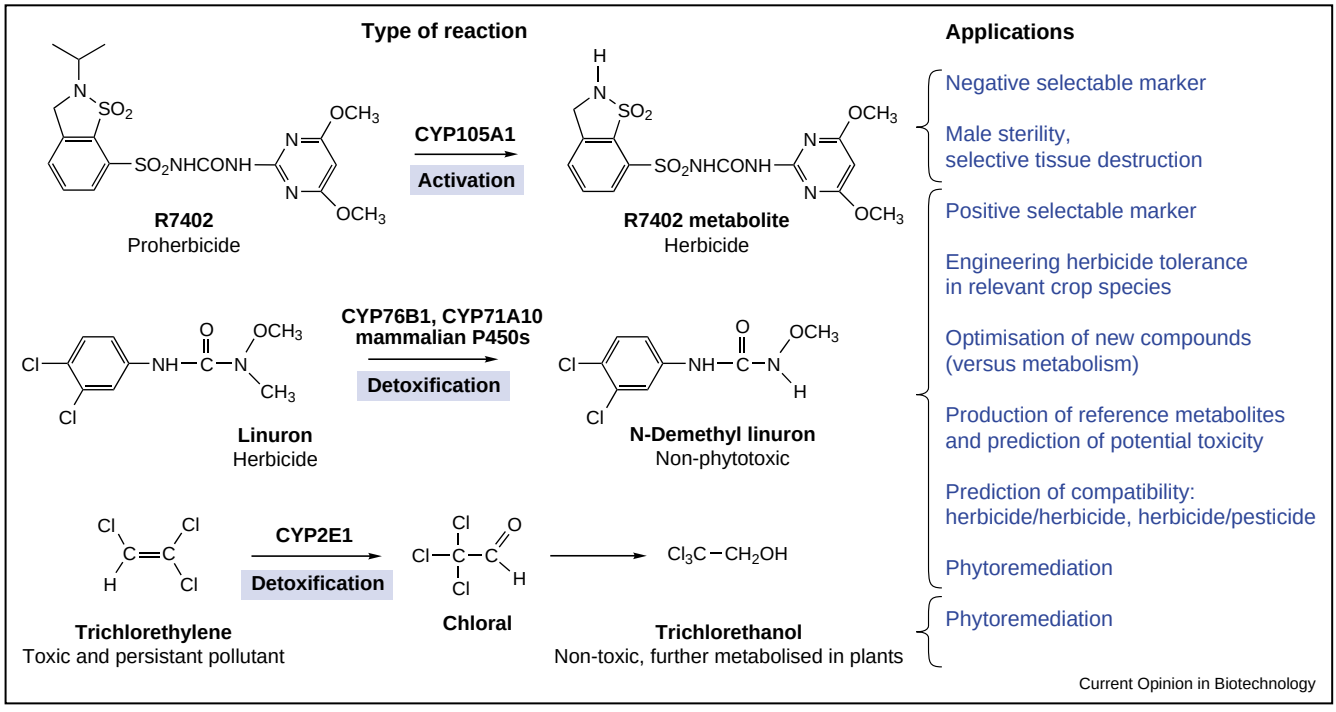
Figure 2



Transfer of the entire pathway for dhurrin synthesis from *S. bicolor* to *A. thaliana* confers resistance to crucifier-specialist flea beetles. **(a)** The dhurrin biosynthetic pathway: CYP79A1 catalyses the conversion of tyrosine to *p*-hydroxyphenylacetaldoxime, which is converted to *p*-hydroxymandelonitrile by CYP71E1. The final step is a glucosylation catalysed by UGT85B1. **(b)** Phenotypes of wild-type and a dhurrin-producing transgenic line expressing the three *S. bicolor* genes CYP79A1, CYP71E1 and UGT85B1. **(c)** Free choice assay between wild type (wt) and a transgenic line accumulating 4% dhurrin/gram dry weight. **(d)** Flea beetle, *Phyllotreta nemorum*.

Plant P450s have more restricted substrate specificity than animal enzymes and have the potential to metabolise herbicides with higher selectivity and turnover rates. Some plant P450s were recently expressed in tobacco or *A. thaliana* and shown to confer increased herbicide tolerance [70,80•]. The tolerance level achievable with the most effective of these enzymes relies on several factors: the number of catalytic steps needed to accomplish a complete detoxification; the rates of metabolism of each herbicide; and the initial tolerance of the transformed plant [80•]. Once herbicide metabolism was obtained by introduction of *CYP76B1*, the products formed were further metabolised by the plant. A 20-fold increase in herbicide tolerance was obtained without

Figure 3



Cytochromes P450 as tools for engineering the metabolism of xenobiotic compounds. Cytochromes P450 can catalyse both the activation and the detoxification of environmental chemicals. Activation can lead, for example, to the conversion of a proherbicide into an active compound, as in the case of CYP105A1 with the sulfonylurea R7402, or to the formation of carcinogens from polyaromatic hydrocarbons. Detoxification of most classes of herbicides in major crops and weeds involves P450 enzymes. Only a few of them, mainly CYP76B1 and CYP71A10, are characterised so far. They catalyse the dealkylation and/or the hydroxylation of phenylurea into non-phytotoxic products. The same reactions can be carried out by mammalian enzymes. Effective phytoremediation has so far only been achieved with a mammalian P450 transgene. *CYP2E1* expression allowed transgenic tobacco to take up and metabolise the pollutant trichlorethylene.

obvious impact on plant fitness. Such a transgene can thus be used as a selectable marker of plant transformation or for bioremediation.

Experimental documentation for valid application of plant P450s for bioremediation is still lacking. However, a mammalian CYP2E1 known to efficiently metabolise trichlorethylene and ethylene bromide has been overexpressed in tobacco plants, resulting in a dramatic increase in uptake and metabolism of both compounds [81••]. As observed with herbicides, P450 metabolites were further processed in the plant.

P450s: potential targets for herbicides and growth regulators?

Genetical approaches have recently demonstrated that some plant P450s serve essential functions in plant growth and development. These P450s may be required for biosynthesis of hormones such as brassinosteroids or essential structural components (e.g. P450s in the upstream phenylpropanoid pathway [37,82]). Such P450s are potential targets for the development of new herbicides or growth regulators [83–88]. Others, involved in the biosynthesis of cutins [89••] are potential targets for the control of herbicide permeability.

Conclusions and future prospects

The extensive analysis of a few representative biosynthetic pathways has recently demonstrated the importance of cytochrome P450 cascades not only for controlling substitution patterns and further acylation of olefins and other natural compounds, but also for building complex ring systems and polymeric structures out of different subunits. Functional characterisation of an increasing number of P450 genes offers the potential to improve the production of existing metabolites and to obtain 'unnatural' natural products with altered substitution patterns and composed of different building blocks. Efficient methods are available to isolate the desired P450 genes [11••,31,73], to express them in relevant plant species or lines [90•], and to improve catalysts for specific functions [91•] or for drug delivery to their absorption or reaction sites [92•]. A combination of these tools and approaches provides an enormous resource for the development of new and more efficient drugs and bioactive compounds and for engineering plant defence. The

detoxification and/or remediation potential of plant P450s still has to be explored, but preliminary results obtained with animal enzymes indicate promising avenues.

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- • of special interest
- •• of outstanding interest

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