



Review

# The Role of Insect Cytochrome P450s in Mediating Insecticide Resistance

Min Ye <sup>1,2</sup>, Bidhan Nayak <sup>3</sup>, Lei Xiong <sup>1,2</sup>, Chao Xie <sup>1,2</sup>, Yi Dong <sup>1,2,\*</sup>, Minsheng You <sup>1,2</sup>, Zhiguang Yuchi <sup>3,\*</sup>  and Shijun You <sup>1,2,\*</sup> 

- <sup>1</sup> State Key Laboratory for Ecological Pest Control of Fujian and Taiwan Crops, Institute of Applied Ecology, Fujian Agriculture and Forestry University, Fuzhou 350002, China; 3190231045@fafu.edu.cn (M.Y.); 2190203005@fafu.edu.cn (L.X.); 1180203026@fafu.edu.cn (C.X.); 1190203004@fafu.edu.cn (Y.D.); msyou@fafu.edu.cn (M.Y.)
- <sup>2</sup> International Joint Research Laboratory of Ecological Pest Control, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou 350002, China
- <sup>3</sup> School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China; bidhannayak@tju.edu.cn
- \* Correspondence: yuchi@tju.edu.cn (Z.Y.); sjyou@fafu.edu.cn (S.Y.)

**Abstract:** In many organisms, cytochrome P450 enzymes are the primary detoxifying enzymes. Enhanced P450 activity can be mediated by the emergence of new genes, increased transcription due to mutations in the promoter regions, changes in enzyme structures and functions due to mutations in protein-coding regions, or changes in post-translational modifications; all of these changes are subject to insecticide selection pressure. Multiple signalling pathways and key effector molecules are involved in the regulation of insect P450s. Increased P450 activity is a key mechanism inducing insect resistance. Hence, downregulation of selected P450s is a promising strategy to overcome this resistance. Insect P450 inhibitors that act as insecticide synergists, RNA interference to induce P450 gene silencing, and the use of transgenic insects and crops are examples of strategies utilized to overcome resistance. This article reviews the latest advances in studies related to insect P450s-mediated agrochemical resistance, with focuses on the regulatory mechanisms and associated pest management strategies. Future investigations on the comprehensive regulatory pathways of P450-mediated detoxification, identification of key effectors, and downregulation strategies for P450s will ecologically, economically, and practically improve pest management.

**Keywords:** cytochrome P450; insecticide resistance; molecular mechanism; effector; signalling pathway; pest management



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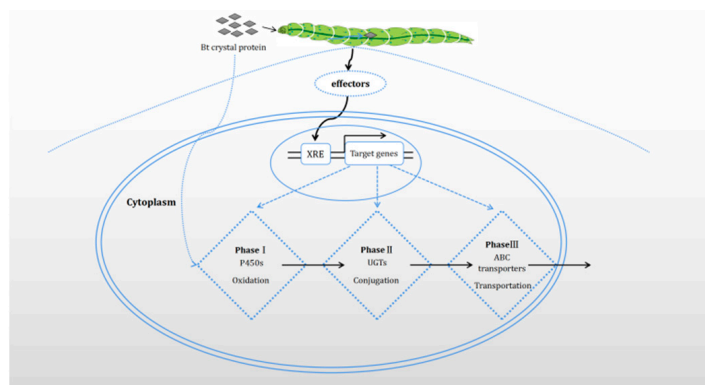
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## 1. Introduction

Management of insect pests predominately relies on synthetic chemical insecticides. Due to their widespread use, there has been a selective effect on insects, leading to the development of insecticide resistance. Five primary resistance mechanisms have previously been reported in insects. These are metabolic resistance, gene target resistance, epidermal penetration resistance, behavioural resistance, and intestinal commensal bacterial resistance. Metabolic resistance comprises three stages. The first stage involves the cytochrome P450 (CYP) enzyme system and other detoxifying metabolic enzymes that catalyse the oxidation reaction of insecticides; the second stage involves uridine diphosphate (UDP) glucosyltransferases (UGTs), which form various conjugated metabolites through conjugation; finally, in the third stage, the ATP-binding cassette transporters (ABC transporters) are involved in eliminating the second-stage metabolites from cells (Figure 1).



**Figure 1.** Schematic diagram of the first, second, and third stages of the insecticide metabolism system. Phase I is mainly involved in oxidation by P450s. Phase II forms a conjugate structure with the products of Phase I through UGTs. The ABC transporters in Phase III transfer the metabolites of Phase II from the cell. Insecticide ligands and effectors mediate the regulation of target genes by binding to xenobiotic response element (XRE) at different stages.

P450s are found in diverse organisms ranging from bacteria to humans [1], that can catalyse a vast array of biochemical reactions including hydroxylation, O-dealkylation, epoxidation, and N- and S-oxidation, and metabolize structurally diverse substrates, including endogenous compounds (hormones, fatty acids, and the hormone hormones, etc.) and exogenous toxic substances (plant secondary biomass and insecticides, etc.). As with other animal CYPs, insect P450s are approximately 500 amino acids in length with a typical recognition feature of FxxGxRxCxG in the heme-binding domain [2]. There are four P450 clans (CYP2, 3, 4, and mitochondrial CYPs) that are present in insects [3], and members of these clans are involved in insect development, growth, and metabolism [4]. Genes in the CYP2 family are evolutionarily conserved [5], and play a role in the development of insect sensory organs [6] and ecdysone synthesis [7,8]. The CYP3 clan is divided into two relatively conservative families, namely CYP6s and CYP9s [9]. These mediate insecticide resistance and xenobiotic metabolism, some of which can be induced by insecticides and phenobarbital. CYP4 genes encode both inducible and constitutive isozymes, which metabolize pheromones and xenobiotics and are involved in odour production [10]. CYP3 and CYP4 genes are present in large numbers in insects, allowing allow them to cope with multiple environmental stresses such as activating moulting by generating ecdysteroid pulses, while the other has evolved genes with taxon-specific paralogous CYPs [8]. Most insect species exhibit comparable numbers of CYPs in their mitochondrial clans. Many members of this enzyme family play important roles in plant-animal warfare, as plant P450s are involved in the production of secondary metabolites that act as chemical defences, while insect P450s metabolise the same compounds to avoid toxicity. Therefore, insect P450s also provide a defensive role against xenobiotics, by participating in or interfering with the specific activation of insecticide precursors, affecting insecticide selectivity [11].

Frequent use of insecticides inevitably leads to resistance in insects involving P450s. Over the past decade, due to the development of molecular and bioinformatics technologies, significant progress has been made in the identifying of P450 genes in resistant insects and associated regulatory mechanisms mediated by P450 genes. This article systematically reviews the characteristics and molecular mechanisms of P450s in mediating insecticide resistance, with a focus on the cascade reaction that regulates the expression of P450s in signal pathways. In addition, proposed resistance management strategies are summarized, with suggestions for future pest control.

## 2. P450-Mediated Insecticide Resistance

P450-mediated metabolic resistance to agrochemicals is universally present in insects and has been reported for many insecticides [12], such as pyrethroids [13], neoni-

cotinoids [14], organophosphates [15], and organochlorines [16]. Increased metabolism by P450s has also been reported to influence the development of cross-resistance to insecticides [17].

Overexpression of CYP9J28 and CYP4G16 mediates resistance to pyrethroids in *Aedes aegypti* and *Aedes albopictus*, respectively [18,19]. Transcriptomic analysis of *Bombyx mandarina* suggests that CYP306A mediates ecdysteroid production and insecticide metabolism [20]. Zhao et al. [21] reported that upregulation of CYP324A12, CYP321F3, and CYP9A68 expression may be involved in the development of resistance to triazophos in *Chilo suppressalis*. The P450 gene CYP6ER1 in field populations of *Nilaparvata lugens* was significantly overexpressed to induce resistance to trifloxystrobin and other traditional agrochemicals [22], while the overexpression of CYP4C71 could metabolize imidacloprid in *Laodelphax striatellus* [23]. In *Plutella xylostella*, expression of CYP321E1 and CYP6BG1 was upregulated in strains resistant to permethrin and chlorantraniliprole, respectively [24].

In addition, RNA interference (RNAi)-based studies have identified several cases of insecticide resistance involving P450s. For example, CYP6CX4 is connected with the resistance to flupyrrolidone in *Bemisia tabaci* [25]; CYP6CY14, CYP6DC1, and CYP6CZ1 are associated with *Aphis gossypii* resistance to acetamiprid [26]; CYP321A8, CYP321A9, and CYP321B1 are responsible for *Spodoptera frugiperda* resistance to chlorantraniliprole [27]. In *Drosophila melanogaster*, Cyp4g1, Cyp6g1, and Cyp12d1 play major roles in reducing DDT sensitivity [28].

### 2.1. Cross Resistance

Cytochromes P450 have a wide range of substrates and, consequently, metabolic resistance often causes insects to develop cross-resistance to other insecticides.

In southern Africa, CYP6AA1 in *Anopheles funestus* was found to mediate the metabolism of various types of pyrethroids and dioxacarb [29], while the overexpression of CYP6Z1 is associated with cross resistance against pyrethroids and carbamates [30]. In *Anopheles gambiae*, overexpression of CYP6P3 has been shown to be responsible for cross resistance to pyrethroid and carbamate [31], and CYP6M2 is linked to cross resistance of pyrethroids and organophosphates [32–35]. Lees et al. [36] found that pyrethroid-resistant strains of *A. gambiae* could also metabolise complex type I inhibitors such as fenazaquin, pyridaben, tolfenpyrad, and fenpyroximate. CYP9K1 can metabolise deltamethrin and pyriproxyfen but not bendiocarb [37]. Zhou et al. [38] found that CYP3A overexpressed in *Sogatella furcifera* under the stress of imidacloprid, deltamethrin, and triazophos. In Sichuan, China, *S. furcifera* was found to be cross-resistant to chlorpyrifos and sulphur-ethyl, mostly due to its overexpression of CYP6ER4 [39]. The overexpression of CYP6ER1 is associated with resistance of *N. lugens* to the first three generations of neonicotinoid insecticides (imidacloprid, thiamethoxam, and dinotefuran) [40], and was subsequently found that the overexpression of this gene is cross-resistant to nitenpyram, clothianidin, sulfoxaflor, cycloxyaprid, ethofenprox, and isoprocarb [41–43]. For *B. tabaci*, researchers found that CYP6CM1 could metabolise neonicotinoid insecticides and pymetrozine [44,45], and its high expression also showed resistance to plant defence metabolites, such as nicotine [46]. In Xinjiang, China, four P450 genes in *B. tabaci* were found to be resistant to imidacloprid and acetamiprid [47]. In China, a chlorantraniliprole-resistant strain of *P. xylostella* is also resistant to flubendiamide, cyantraniliprole, and abamectin [48]. In addition, *P. xylostella* in central China is highly resistant to indoxacarb and cross-resistant to metaflumizone, beta-cypermethrin, and chlorfenapyr [49].

Asymmetric cross-resistance was first proposed for *Pectinophora gossypiella*. The laboratory-selected *P. gossypiella* strain resistant to Cry2Ab is cross-resistant to Cry1Ac, but the laboratory-selected strain resistant to Cry1Ac showed no cross resistance to Cry2Ab [50]. This phenomenon is referred to as “asymmetric cross-resistance” [50]. *Helicoverpa armigera* also exhibits asymmetric cross-resistance for these two toxins. The asymmetric cross resistance of *P. xylostella* is reflected in a tebufenozide-resistant strain that is cross-resistant to abamectin, while an abamectin-resistant strain is not resistant to tebufenozide [51]. CYP4M7

and *CYP6K1* induce tebufenozide and abamectin resistance, respectively. The selection of tebufenozide enhanced *CYP4M7* expression as well as *CYP6K1* expression; conversely, results from a resistance experiment of an anti-abamectin strain to tebufenozide showed that *CYP6K1* expression was increased, whereas *CYP4M7* expression was downregulated with no cross-resistance observed [52].

## 2.2. Evolutionary Adaptability

Evolutionary adaptability is also known as biological adaptability. Even under the stress of the same insecticides in different populations of the same insects, the resistance-related cytochrome P450s evolutionarily selected differ. A specific P450 may be resistant to multiple insecticides, or two or more P450s may be resistant to one insecticide. Therefore, resistance is a characteristic of biological evolution.

RNA sequencing transcription profile analysis of mosquitoes throughout Africa found that *CYP9K1* is highly overexpressed in Uganda [53], *CYP6P5* and *CYP6P4a/b* are both overexpressed in Ghana, *CYP325A*, *CYP9J11* and *CYP6N1* is overexpressed in Cameroon, while *CYP315A1* is overexpressed in all locations except Malawi [54,55]. Subsequently, excessive transcription of P450s were also found in resistant populations of *A. aegypti* [56], and *CYP9J28* and *CYP6BB2* were frequently detected in research on pyrethroid resistance [57]. Conversely, *CYP9J28* showed the highest resistance to pyrethroids in Pakistan [19], while *CYP9J32* showed the highest resistance to deltamethrin and cypermethrin in Thailand, Mexico, and Vietnam, *CYP9J24* and *CYP9J26* had the highest resistance to pyrethroids in Latin America, and *CYP9M6* and *CYP4D24* mediates resistance to pyrethroids in Asia [58,59]. Different *Drosophila* strains utilize different genes in DDT resistance. Canton-S (susceptible strain) and 91-R (resistant strain) are mediated by *Cyp6g1*, and *Cyp6a2*, while *Cyp12d1* is only overexpressed in 91-R and Wisconsin (resistant strains) [60–62]. *CYP6AY1* and *CYP6ER1* are the main reasons for *N. lugens* and *S. furcifera* resistance to imidacloprid [63], among which, *CYP6AY1* can effectively metabolise imidacloprid, and the expression of *CYP6ER1* increases under the influence of imidacloprid [64]. The resistance of *L. striatellus* to deltamethrin is related to the oxidative and detoxification effects of *CYP6FU1* and *CYP439A1v3* [65,66]. Overexpression of multiple P450 genes is associated with deltamethrin resistance of *H. armigera* in field lines with different resistance levels in West Africa [67], and the high-level of resistance in *H. armigera* from different field strains in northern China to fenvalerate is also related to multiple P450 genes, as *CYP332A1*, *CYP6B7*, and *CYP9A12* were found to be overexpressed in all field strains [68–70]. Shi et al. [71] found that all members of the *CYP6AE* subfamily, except *CYP6AE20*, could effectively convert fenvalerate to 4'-hydroxyfenvalerate.

## 3. Molecular Mechanisms of P450-Mediated Insecticide Resistance

Increased cytochrome P450 activity is associated with the development of insect resistance, as reflected by the increased enzyme expression due to new gene evolution or promoter region mutations affecting transcription, changes in the amino acid sequence of protein-coding regions, or changes in enzyme functions due to post-translational modifications such as phosphorylation changes, usually generated under selective pressure from insecticides.

### 3.1. Upregulation of Enzyme Expression

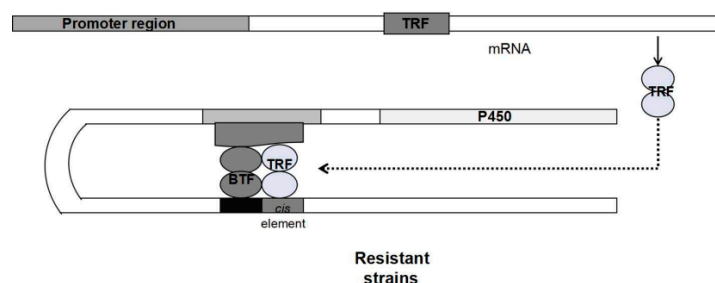
Increased enzyme expression can be attributed to changes in the gene copy number caused by the evolution of new genes, such as through gene amplification or gene duplication, and the influence of *cis*-acting elements and *trans*-regulatory factors of transcriptional regulation in the promoter region.

In *Drosophila virilis*, which has multiple copies of *Cyp6g1*, one copy confers DDT resistance while another confers nitenpyram resistance, which indicates that the protein sequence differences between the copies after replication affect enzyme activity [72]. The *CYP6A5* and *CYP6A5v2* alleles from the pyrethroid-resistant strain of *Musca domestica* have

extremely high amino acid sequence similarities [73]. Overexpression of the *CYP6CM1* gene in *B. tabaci* is closely correlated with imidacloprid resistance, while carriers of the *r-Q* allele (*CYP6CM1* allele of *B. tabaci* resistance Q biotype) are more resistant to imidacloprid [74].

Cases of P450 gene amplification are limited; both heterogeneous replication and homogeneous amplification of CNV (copy number variation) in *A. gambiae* mediates insecticide resistance [75,76]. However, gene amplification is more common in *A. aegypti*. For example, *CYP9M6* gene amplification has been found in permethrin-resistant populations [75,77]. In Laos, the CNV of *CYP6BB2* and *CYP6P12* in *A. aegypti* may be related to pyrethroid and DDT resistance [78], while the overexpression of *CYP9J* in pyrethroid-resistant strains in the Caribbean is partly attributed to gene amplification [79].

Transcriptional regulation, the most important mechanism involved in the regulation of enzyme expression in eukaryotes, is mostly achieved through complex interaction of basal transcriptional elements (BTF), *cis*-acting elements and the *trans*-regulatory factors (Figure 2). The increase in *Cyp6g1* gene expression in *D. melanogaster* correlates with the long-terminal repeat of the *Accord* retrotransposon inserted upstream of the transcription initiation site [80–82], and it was later discovered that the induction of *Cyp6a8* by caffeine, phenobarbital (PB) and DDT was mediated by *cis*-acting elements located in -11/-199 DNA [83], while the transcription factor Nrf2/Maf binding site in the core sequence of 5'-promoter enhanced the constitutive transcription of *Cyp6a2* and raised the resistance to DDT [84,85]. A novel alternative splicing site in the 5'-untranslated region of *CYP6AY1* in *N. lugens* leads to increased activity of the *CYP6AY1* promoter and causes enhanced resistance to buprofezin and imidacloprid [86], and then it was found that an alternative 5'-UTR in *CYP6ER1* confers resistance to imidacloprid in *N. lugens*, and this newly discovered transcript used different transcription initiation sites in the alternative promoter compared with the previous transcript [87,88]. The promoter activity of *CYP6FU1* in a deltamethrin-resistant strain of *L. striatellus* is upregulated via multiple *cis*-acting elements [89]. The induction of *H. armigera* *CYP321A1* also requires multiple *cis*-acting elements to be mediated [90]. Subsequently, the *cis*-acting elements of *CYP6B7* induced by 2- tridecanone in the *CYP6B7* promoter were found to be located between -280 and -257 bp [91]. CncC and Maf transcription factors are not only responsible for P450-mediated fenpropathrin metabolism in *Tetranychus cinnabarinus* [92], but also mediate the P450-related resistance to imidacloprid in potato beetles, *Leptinotarsa decemlineata* [93]. In addition, the overexpression of four CYP (*CYP6BB2*, *CYP6Z8*, *CYP9M5*, and *CYP9M6*) genes in *A. aegypti* is caused by *trans*-regulatory factors [94]. *CYP6D1* in *M. domestica* is also regulated by a *trans*-regulatory factor, and its expression is induced in response to the prototype P450 inducer phenobarbital (PB) in strains susceptible to insecticides [95]. The transcription factor FTZ-F1 and the *cis*-acting elements are involved in the overexpression of *CYP6BG1* in chlorantraniliprole resistant *P. xylostella*, and the *cis*-acting element may be located in the -680 downstream region of the promoter [96].



**Figure 2.** A speculative model of P450 gene regulating insecticide resistance in resistant strains. The interaction of P450 gene in resistant strains with basic transcription factor (BTF) and *cis*-acting element(*cis*- element) or *trans*-regulatory factor (TRF) in insecticide resistance [97].

In addition, it has been reported that some insecticides are activated upon metabolism by P450s, therefore the downregulation of the expression of these CYPs leads to



resistance. Some P450 enzymes have been reported to mediate the oxidation of organophosphate insecticides into more toxic structures that inhibit acetylcholinesterase. In the presence of organophosphate insecticides, it could be a good strategy for the survival of insects to reduce the expression levels of CYP genes [98]. Subsequently, in *Chironomus tentans*, the chlorpyrifos activity is increased due to the enhancement of the oxidation process from chlorpyrifos, to chlorpyrifos oxygenation via *CtCYP6EX3*. When compared with the control larvae, the mortality of *C. tentans* larvae to chlorpyrifos and the mixture of atrazine and chlorpyrifos decreased by 24.1% and 20.5%, respectively, after the expression of *CtCYP6EX3* was partially inhibited (53.1%) [99].

However, the production of new active P450s can also lead to resistance. Multiple recombination at two P450 (*CYP337B2* and *CYP337B1*) sites led to the overall resistance of *H. armigera* to pyrethroids [100]. The resistance of the Australian *H. armigera* strain to fenvalerate originates from the special P450 chimeric enzyme *CYP337B3* produced by an unequal cross between two parental P450 genes, *CYP337B2* and *CYP337B1*, that can metabolize fenvalerate to 4'-hydroxyfenvalerate, which is not toxic to susceptible larvae. The presence of *CYP337B3* in this strain of resistant insects alone confers resistance to fenvalerate [101]. In addition, this mechanism also exists in the cypermethrin-resistant strains of Pakistan. *CYP337B3* has independently evolved twice through unequal crosses between *CYP337B2* and two different *CYP337B1* alleles, with resistance to cypermethrin being approximately 7-fold [102]. In Brazil, chimeric *CYP337B3* is the source of pyrethroid resistance in the Brazilian *H. armigera* population [103]. However, in the Chinese *H. armigera* population, P450-mediated fenvalerate resistance level variation is unrelated to the *CYP337B3* genotype [104]. In addition, polymorphic *Cyp12a4/Cyp12a5* chimaeras were only found in *Drosophila* and are involved in insecticide resistance [105].

### 3.2. Changes of Enzyme Functions

P450-mediated insecticide resistance may also be related to changes in the amino acid sequence in the protein-coding regions or post-translational modifications of the protein, such as phosphorylation, thereby enhancing the activity of the related detoxifying enzymes.

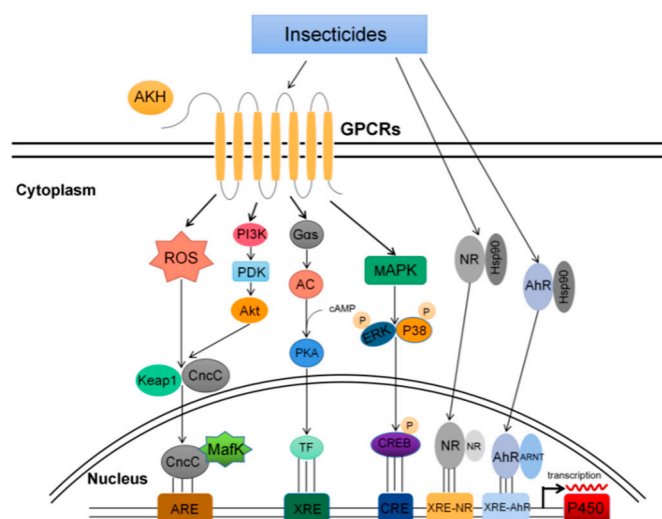
The protein truncation of three P450s (*Cyp6a2*, *Cyp316a1*, and *Cyp6a14*), resulting from fixed non-synonymous mutations, has been identified in the *D. melanogaster* strain resistant to DDT [106], and three point mutations of *Cyp6a2* are considered to be responsible for 1,1,1-trichloro-2,2-bis-(4'-chlorophenyl)ethane (DDT) resistance in the RDDT<sup>R</sup> strain of *D. melanogaster* [107]. Three amino acid changes in the *CYP6P9b* resistance alleles in *Anopheles* cause the pyrethroid resistance mutations [55,108]. In addition, the polymorphism analysis of *CYP6Z1* and *CYP6Z3* showed 19 amino acid changes in *CYP6Z1* and 9 amino acid changes in *CYP6Z3*, which identified potential mutations related to pyrethroid resistance in *Anopheles* [109]. It has recently been discovered that the overexpression of a variant *CYP6ER1* with amino acid substitutions and deletions in *N. lugens* populations in East and Southeast Asia is associated with imidacloprid resistance [110].

Insect P450s-mediated resistance is also related to phosphorylation regulation, a type of protein post-translational modification (PTM) [111]. Phosphorylation is involved in almost all biological processes [112]. In eukaryotic cells, approximately one-third of the proteins are modified by phosphorylation. The addition or removal (dephosphorylation) of phosphate groups may activate or inactivate the target protein, thereby regulating the related biological activities [113,114]. Previous studies have shown that phosphorylation is involved in almost all life processes including signal transduction, cell membrane function, mitochondrial function, transcription, cell cycle [115–118]. However, there are not many reports on insect phosphorylation-mediated resistance. One example is known for regulation of acetylcholine receptor (nAChR) signaling, where resistance to imidacloprid is regulated by intracellular phosphorylation [119]; *P. xylostella*-specific PKA phosphorylation sites may regulate insect ryanodine receptor function [120]. There are limited cases of P450 regulation. An example is that, when *Bombyx mori* is exposed to chlorantraniliprole, Akt in the PI3K pathway in vivo may decrease Keap1 expression and increase CncC expression

due to dephosphorylation. Thus, the P450 enzyme activity downstream of the pathway is significantly increased, thereby enhancing detoxification [121]. CYP6CM1-mediated imidacloprid resistance in *B. tabaci* directionally activates CREB protein through ERK and p38 factor phosphorylation in the MAPK pathway, and subsequent binding to the CRE site results in the upregulation of the expression of this gene [122]. Recently, it has been reported that this pathway can also mediate the differential expression of downstream genes through the phosphorylation of related proteins in *P. xylostella*, making it highly resistant to *Bacillus thuringiensis* (Bt) insecticidal protein [123].

#### 4. Effector Molecules and Signal Pathways of P450s Expression Regulation

Recently, to better understand the detoxification and metabolism of P450s, research has shifted from initial functional identification to upper and lower regulation cascade reactions. An increasing number of signal pathway regulation pathways and important effector molecules in the pathways have been discovered, which provided new ideas to improve our understanding of the potential resistance mechanisms of insects and to help formulate more specific and efficient control measures. The following is a summary of the reported regulatory pathways (Figure 3).



**Figure 3.** Model of the theoretical signalling pathway for insecticide resistance induced by insect P450s. Some insecticides can be sensed by the membrane receptor GPCR, which then stimulates downstream effectors to bind to specific sites in the cytoplasm and nucleus that trigger compensatory responses that affect P450 activity; other insecticides can diffuse into the cell and be transferred to the nucleus by NR or AhR in the cytoplasm binds to nuclear receptor sites. The black arrow indicates the cascade of effectors in the signalling pathway. AKH, adipokinetic hormone; GPCRs, G protein-couple receptors; ROS, reactive oxygen species; Keap1, Kelch-like ECH-associated protein 1; CncC, Cap'n' Collar isoform C; MafK, Musculoaponeurotic fibrosarcoma K; ARE, Antioxidant responsive element; PI3K, phosphoinositide-3-kinase; PDK, 3-phosphoinositide-dependent kinase; Akt, protein kinase B; Gas, G protein alpha unit which stimulates adenyl cyclase; AC, adenyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; TF, Transcription factor; XRE, xenobiotic response element; MAPK, mitogen-activated protein kinase; ERK, extracellular regulated protein kinase; P38, P38 mitogen-activated protein kinase; CREB, c-AMP response element binding protein; -P, phosphorylation; CRE, cAMP response element; NR, nuclear receptor; AhR, aryl hydrocarbon receptor; Hsp90, heat shock protein 90; ARNT, aryl hydrocarbon receptor nuclear translocator; XRE-NR, xenobiotic response element-nuclear receptor; XRE-AhR, xenobiotic response element-aryl hydrocarbon receptor.

#### 4.1. GPCRs Pathway

G protein-coupled receptors (GPCRs) are an important family of transmembrane proteins that transmit extracellular ligand signals to specific intracellular signalling pathways through the cell membrane thus playing an important role in the regulation of insect biology, physiology, and behaviour. Investigation of GPCRs is providing new directions for insecticide development [124–127].

Ma et al. [128] detected the gene expression profiles of three *M. domestica* strains and found that when compared with the control strain, five GPCR genes were upregulated in N-IRS (imidacloprid-resistant near-isogenic strains) and eight in IRS (imidacloprid-resistant field population). The transgenic strains of *D. melanogaster* with GPCR genes have significantly upregulated tolerance to imidacloprid and increased expression of the cytochrome P450 genes [128]. Cao et al. [129] cloned the full length of a methylcellulose-like GPCR gene (*Ldmthl1*) from *Lymantria dispar* and described the secondary and tertiary structures of *Ldmthl1*. RNAi with *Ldmthl1* leads to a decrease in the resistance of *L. dispar* to deltamethrin and inhibits the expression of the downstream gene P450 [129]. Subsequently, Sun et al. [130] studied the physiological functions of *L. dispar* ocular albinism type 1 (OA1), and used transgenic technology to overexpress the gene of *L. dispar* (*LdOA1*) in *D. melanogaster*. The deltamethrin exposure test showed that the increased tolerance of transgenic *D. melanogaster* to deltamethrin when compared with the control strain was due to the significant upregulation of *LdOA1* expression and the upregulation of downstream P450 genes, suggesting that *LdOA1* might be involved in P450-mediated detoxification metabolism [130]. NYD-OP7, a mosquito opsin gene within *Culex pipiens pallens*, mediates moderate resistance to deltamethrin. Knockdown of NYD-OP7 and its downstream effector phospholipase C (PLC) can increase the susceptibility of *Cx. pipiens pallens* to deltamethrin and reduces the expression of P450 genes [131,132]. Li et al. [133] reported for the first time that the expression of P450 genes is mediated by GPCR-related genes in *Culex quinquefasciatus*. When the four upregulated GPCR-related genes, (*CYP6AA7*, *CYP9M10*, *CYP9J34*, and *CYP9J40*) in the high-resistance *Cx. quinquefasciatus* strain were inhibited by RNAi, the resistance to permethrin was reduced [133]. Further research found that interference with the rhodopsin-like GPCR gene in *Cx. quinquefasciatus* decreased the expression of two protein kinase A genes (PKAs) and four resistance-related P450 genes, thereby decreasing permethrin resistance [134]. Subsequently, the downstream effectors G $\alpha$ s and ACs of the GPCR signalling pathway could also reduce the expression of PKA and the resistant P450 genes after interference, resulting in an increase in the sensitivity of *Cx. quinquefasciatus* to permethrin, and revealing that the GPCR/G $\alpha$ s/AC/cAMP- PKA-mediated regulatory pathways control P450 gene expression in *Cx. quinquefasciatus* [135]. This regulatory pathway has also been identified in field collected and laboratory-susceptible *Cx. quinquefasciatus*, indicating the general role of the GPCR regulatory cascade in the susceptibility of *Cx. quinquefasciatus* to insecticides, and its influence on the development of resistance through P450-mediated detoxification [136]. Overall, these results demonstrate the universal regulatory role of GPCR/G $\alpha$ s/AC/PKA in insecticide resistance by showing that GPCR/G $\alpha$ s/AC/PKA can regulate the expression of P450 genes related to insecticide resistance in insects such as *Cx. quinquefasciatus*, *D. melanogaster*, and *S. frugiperda* [137].

#### 4.2. MAPK Pathway

The mitogen-activated protein kinase (MAPK) pathway is a conserved serine/threonine protein kinase that responds to most extracellular stimuli and controls processes such as cell proliferation, apoptosis, and movement [138]. In vitro and in vivo experiments using *B. tabaci* by Yang et al. [122] showed that the ERK and p38 factors in the MAPK pathway are activated by the phosphorylation of the 111 serine of the CREB protein. The CREB protein then activates *CYP6CM1* by binding to the CRE-like site in the promoter of this gene, leading to an increase in its expression and resistance to imidacloprid [122].



#### 4.3. PI3K Pathway

The PI3K pathway mediates cell proliferation, survival, and signal transduction through the phosphorylation of multiple substrates, including related kinases, signal proteins, and transcription factors [139]. Mao et al. [121] continuously fed *B. mori* larvae with chlorantraniliprole-impregnated mulberry leaves and found that the transcription levels of PI3K, PDK, Akt, CncC, and Keap1 in the PI3K signalling pathway were significantly upregulated. However, Akt was inhibited at the protein level, which decreased the expression of Keap1 and increased that of CncC and P450 enzyme activity downstream of the pathway [121].

#### 4.4. CncC Pathway

*Drosophila* Nrf2 orthologous CncC is the central regulator of the detoxification reaction of heterologous organisms. Changing the operation of CncC or its negative regulator Keap1 will lead to the expression of xenobiotic-inducible genes; interruption of Keap1 function in any major metabolic organ will lead to significant resistance to pharmaceuticals [140]. When *Spodoptera litura* was exposed to lambda-cyfluthrin, P450 activity and *CYP6AB12* transcription levels increased. Double-stranded RNA (dsRNA) injection of piperonyl butoxide (PBO) inhibits the activity of P450s and silences *CYP6AB12*, reducing the tolerance of larvae to lambda-cyfluthrin. Lambda-cyhalothrin exposure induced the expression of CncC and Maf and increased the content of hydrogen peroxide and activity of antioxidant enzymes. Intake of the reactive oxygen species (ROS) scavenger N-acetylcysteine can reduce the accumulation of HO, inhibit the expression of CncC, Maf, and *CYP6AB12*, and increase the sensitivity of larvae to lambda-cyfluthrin. Lambda-cyhalothrin has been shown to induce cytochrome P450 *CYP6AB12* by causing ROS bursts and activating the CncC pathway in *S. litura* [141]. Studies have also shown that the anti-stress response of insects induced by insecticides is mediated by adipokinetic hormones (AKHs) [142]. *CYP6ER1* and *CYP6AY1* confer resistance to neonicotinoid imidacloprids in *N. lugens*, and the neuropeptide adipokinetic hormone (AKH) negatively regulates the expression of *CYP6ER1* and *CYP6AY1* and inhibits imidacloprid resistance by inhibiting P450 gene expression. After RNAi and AKH peptide injection, exposure to imidacloprid inhibited the AKH cascade and cause a burst of ROS, but activated the effectors CncC and MafK to induce the expression of *CYP6ER1*, but the pathway of ROS-mediated *CYP6AY1* expression is still unknown [143].

#### 4.5. Nuclear Receptors

Nuclear receptors (NRs) are ligand-dependent transcription factors (TFs) widespread in various organisms. Typically, insects contain 21 genes that encode nuclear receptors. As transcription regulators, they mediate insect development, which has been intensively studied in the model organism *D. melanogaster* [144]. Increasing evidence has shown that the resistance of insects to synthetic insecticides is regulated by nuclear receptors. hormone receptor 96 (HR96) regulates phenobarbital induction of *M. domestica* P450 *CYP6D1* in *Drosophila* S2 cells [145] and the basal expression of DDT metabolism by *CYP6G1* and *GSTE1* in *D. melanogaster* [146]. Lu et al. [147] used RNAi technology to interfere with HR83 in *N. lugens* and found that the expression of four uridine diphosphate glycosyltransferase (UGT) genes, three P450 genes, and four carboxylesterase (CarE) genes were significantly reduced, and the resistance to chlorpyrifos was weakened, indicating the phenomenon of hormone receptor 83 (HR83) mediated resistance. Meanwhile, Cheng et al. [148] discovered that inhibiting hepatocyte nuclear factor 4 (HNF4) could significantly upregulate the expression levels of *UGT-1-7*, *UGT-2B10*, and *CYP6ER1*, and improve the resistance of *N. lugens* to imidacloprid. In addition, the P8 nuclear receptor gene of *Tetranychus urticae* responds to the stress caused by exposure to acaricides. RNAi experiments have shown that the P8 nuclear receptor regulates the activity of P450 enzymes, and that mites are sensitive to acaricides [149]. In *P. xylostella*, the orphan nuclear receptor FTZ-F1 mediates the expression of *CYP6BG1*, which is resistant to chlorantraniliprole [96].

Aromatic hydrocarbon receptors are ligand-activated transcription factors that belong to the nuclear receptor superfamily and are related to HSP90, a molecular chaperone in the cytoplasm [150]. The AhR/ARNT complex can alter transcription by binding to homologous response elements or other transcription factors [151]. The AhR/ARNT pathway in insects has been intensively studied in the *CYP6B1* and *CYP6B4* promoters of *Papilio polyxenes* and *Papilio glaucus* [152–154]. It has also been reported that the tolerance of *A. gossypii* to gossypol and spirotetramat underlying *CYP6DA2*, and, AhR and ARNT mediate the regulation of *CYP6DA2* [155]. It was also subsequently demonstrated that AhR and ARNT conferred nicotinic adaptability to tobacco by *Myzus persicae* through the synergistic regulation of the transcript levels of *CYP6CY3* and *CYP6CY4* [156].

### 5. Management of P450-Mediated Insecticide Resistance

The mechanisms underlying insecticide resistance are complex, and over time, different mechanisms increase the probability of resistance to multiple insecticides as a result of high selective pressure. Therefore, appropriate resistance management strategies are required to overcome insect resistance [157]. Regarding the participation of P450s, the use of pesticide synergists and RNAi-based technology seems to be the most promising approach.

Deployment of a mixture of insecticides with different modes of action to target pests improves the efficacy. The combined use of bifenthrin and abamectin or spinosyn and indoxacarb can significantly increase its toxic potency in insect field populations [158]. However, the combination of bifenthrin and chlorpyrifos showed similar effects when using either of the two insecticides alone. In addition, the combination of insecticides may lead to multiple resistances that are difficult to control, therefore, alternative strategies are required. Piperonyl butoxide (PBO) has been used as an insecticide synergist, long before its inhibitory effects on xenobiotic metabolising enzymes were known [159]. PBO has low specificity for inhibiting P450s in many insects, which may explain its wide application. However, since the P450s of some insects play a more important role in other physiological process besides detoxification, exposure to PBO (or similar compounds) may have toxic effects on useful insects. Therefore, it is advisable that we develop more specific insect P450 inhibitors to further reduce the side effects on other insects while having a stronger selective effect on pests.

The use of RNAi to trigger host-induced gene silencing (HIGS) is considered a potential next-generation pest control strategy [160]. RNAi specifically targets the desired pest species and enhances their sensitivity to phytotoxins and chemical pesticides [161,162], including the expression of pest-targeted dsRNA in transgenic plants to control pest populations [163]. Transgenic tobacco plants have been created to express dsRNA transiently against the dicer gene of the plant and the midgut gene of *Manduca sexta* larvae. The results showed that the plant expressed longer *CYP6B46* dsRNA, which mediates the inhibition of target genes in the larvae and increases toxicity [164]. Similarly, the feeding of *H. armigera* on cotton plants expressing *H. armigera* cysteine protease and P450 genes weakened the barrier effect of the peritrophic matrix (PM), resulting in insects being more susceptible to virus infection [165]. Silencing the *CYP6B6* gene by expressing dsRNA in bacteria, and using these bacteria to feed *H. armigera* larvae can increase larval mortality by 27% [166]. Differences in the efficiency of RNAi at the gene, tissue, and species levels, as well as the lack of reliable dsRNA delivery methods, have limited the development and utilisation of RNAi-based strategies for controlling herbivorous pests [167]. Another limitation is that the cellular uptake process of dsRNA and systemic diffusion into the host can lead to off-target effects [168]. New methods for constructing dsRNA, selection of target genes, and technologies to enhance the stability of dsRNA are therefore underway, with the aim of improving the efficiency of RNAi to improve pest management and provide improved pest control [169].

## 6. Summary and Prospects

This review comprehensively updates information about the involvement of P450s in insecticide resistance, highlights latest findings of molecular mechanisms underlying P450-mediated agrochemical resistance, and provides a reference for future research into the mechanisms of insecticide resistance. The continuous development of bioinformatics and functional research technologies has facilitated the investigation of cytochrome P450-mediated insect resistance, and consequently, an increasing number of P450 genes have been identified and analysed. However, since P450s are complex multifunctional enzyme systems, most studies have been conducted at a descriptive level for metabolism or synergy related to insecticide resistance, and only a few studies have investigated the molecular mechanisms of gene actions. A deeper understanding of the resistance stability, cross-resistance, and detoxification mechanisms of different insect P450s, as well as the insect-resistant strains selected in the laboratory, will help to inhibit or at least slow down the evolution of resistance in field populations of insects. In addition, there is a need to better understand the P450s of beneficial insects, such as bees, to minimize the harmful effects for them. With the discovery of the potential functions of different regulatory pathways in insecticide resistance and their regulatory functions in the expression of resistance-related P450 genes, future research should focus on the upstream and downstream factors of regulatory pathways to identify the key regulatory factors and the interactions between different regulatory pathways. To finally obtain a complete regulatory pathway for insect P450 genes participating in the detoxifying metabolism, the inhibition of other factors while inducing the P450s expression by insecticides also warrants further investigation. Great progress has been made in overcoming resistance to insecticides by using insecticide mixtures or HIGS. In the future, the determination of high-resolution crystal structures of insect P450s would provide more accurate templates for the design of selective inhibitors that specifically target pest P450s. Further development of pest-specific P450 inhibitors, RNAi technology, and genetic-based strategies is expected to improve pest management in a manner that is ecologically more acceptable, economically beneficial, and practical.

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