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Xenobiotic Detoxification in Insects: Regulatory Mechanisms and Evolutionary Roles of Cytochrome P450

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Abstract

Insects have developed sophisticated defense mechanisms to survive in environments rich in xenobiotics, including plant allelochemicals and synthetic insecticides. A major component of their adaptive response is the cytochrome P450 monooxygenase (P450) enzyme system, which plays a central role in the detoxification of these compounds through oxidative metabolism. P450-mediated resistance involves multiple mechanisms, including gene overexpression, allelic variation, and substrate-specific catalytic activity. These enzymes are inducible by both phytochemicals and synthetic insecticides, often leading to cross-resistance. The evolutionary arms race between plants and herbivorous insects has driven diversification in P450 genes, especially in specialists like *Papilio* species and generalists such as *Helicoverpa zea*. Furthermore, the regulation of P450 expression is modulated by complex signaling pathways including the MAPK, ROS/CncC, and AhR/ARNT pathways, with transcription factors like CREB, CncC, and HR96 playing key roles. Understanding these regulatory mechanisms provides insight into how insects sense and respond to xenobiotic stress and offers promising targets for novel pest control strategies. This review highlights the metabolic and regulatory versatility of P450s, their economic importance in insecticide resistance, and underscores the need for further research into the molecular networks governing xenobiotic adaptation in insects.

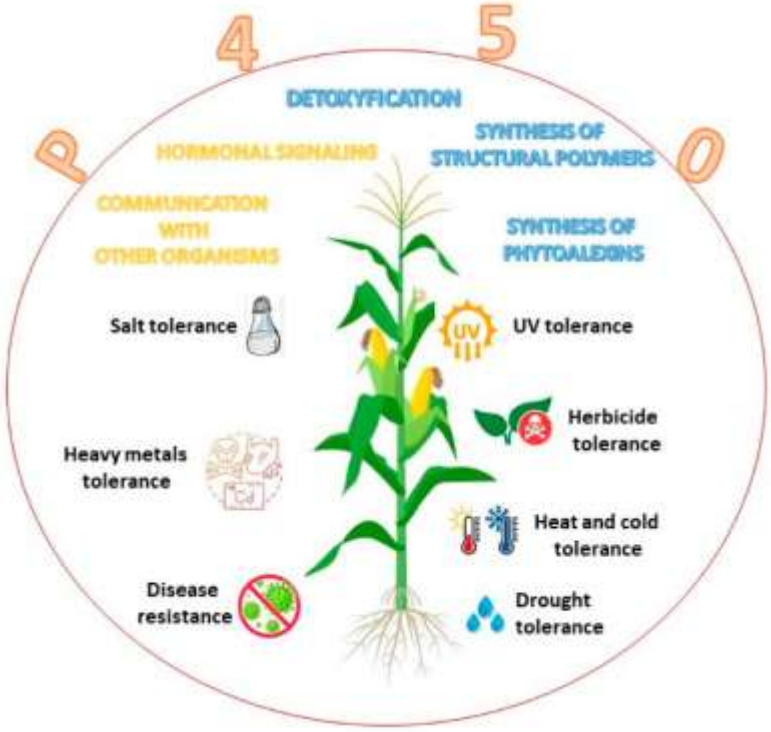
Introduction

Insect resistance to xenobiotics, including naturally occurring plant allelochemicals and synthesized insecticides, is an ongoing pest control concern and a great paradigm for studying microevolution and environmental adaption mechanisms (1). Resistance to xenobiotics is often associated with improvements in the metabolic capacity of detoxification enzymes and decreases in target site sensitivity (2). Identifying genes involved with xenobiotic resistance and structural alterations impacting their activities has made significant progress in the past decade (3). In order to protect themselves from these phytochemicals, insects have evolved a number of adaptive modifications, such as physiological and behavioural modifications that result in xenobiotic resistance, altered penetration, sequestration, target genes mutations, and detoxification (1). In general, three phases are involved in the detoxification of xenobiotics: phase I (oxidation, epoxidation, hydroxylation, N- dealkylation, desulfuration, O-dealkylation, sulfoxidation, reduction and hydrolysis), phase II (sulfate conjugation, glucose conjugation, phosphate conjugation, amino acid conjugation and glutathione conjugation), and phase III (excretion) (5). The addition of functional groups such as hydroxyl, carboxyl, and epoxide occurs during phase I interactions with xenobiotics. This phase I products can be subjected to further conjugation processes with endogenous compounds. These conjugation processes are referred to as phase II reactions. Examples of endogenous compounds include sugars, glutathione, amino

acids, dimethoate, phosphate, and sulphate. Conjugation products are often more polar, less poisonous, and easier to excrete than their parent molecules (6). Among the phase I processes, oxidation is regarded as the most significant. Microsomal cytochrome P450 monooxygenases (also known as mixed-function oxidases (MFO) or microsomal oxidases) perform out the oxidative processes. These enzymes are typically found in mammals, birds, reptiles, fish, crustaceans, molluscs, insects, bacteria, yeast, and higher plants and are present in the endoplasmic reticulum of eukaryotic cells (7). Microsomal monooxygenases comprise three parts: cytochrome P450, NADPH-cytochrome P450 reductase, and a phospholipid (phosphatidylcholine).
Due to their genetic variability and broad substrate selectivity, P450s are known to play vital roles in both endogenous compound metabolism and xenobiotic detoxification, resulting in the development of resistance to synthetic insecticides and facilitating insect adaptation to phytochemicals (8). Increased P450 protein synthesis and enzymatic activity are linked to improved insect xenobiotic metabolic detoxification. Insect P450s induction and continuous overexpression are engaged in xenobiotic detoxification and environmental adaptation. As P450s are upregulated by a diverse range of xenobiotics thus, interactions between natural and synthetic xenobiotics and P450-induced cross-tolerance/resistance are very common (9). Most researchers have concentrated on the discovery and functional study of P450s involved in xenobiotic detoxification, but few have looked at the pathways that regulate them. How insects sense xenobiotic and convert stressful signals into a detoxifying response is still unclear. Filling these information gaps will increase our understanding of P450-mediated insect adaptation to xenobiotic processes while also providing prospective new pest control targets.

P450s and xenobiotic detoxification:
Throughout the long history of insect-plant interactions, plants defend themselves against herbivores using a complex and dynamic defensive mechanism that includes structural modifications, toxic metabolites (phytochemicals), and the attracting parasitoids and enemies of the target pests. (10). Herbivorous insects have evolved various counter-defense mechanisms, including diverse detoxification enzymes, to combat these phytochemicals and adapt to host flora (11). The P450s are primarily responsible for xenobiotic detoxification in insects. P450s, as a superfamily, may catalyze various modification processes, including peroxidation, hydrolysis, oxidation, dehydrogenation, and reduction (12). P450s' catalytic plasticity and broad substrate specificity benefit insects in dealing with a wide range of phytochemicals (13). The abundance of secondary metabolites in host plants is thought to be a primary driving force in developing P450-induced detoxification in both specialized and generalized herbivores (14). Because they consume predominantly specific plant groups, specialist insects have to deal with relatively few specific phytochemicals throughout their life. Compared to generalists, specialist herbivores often have developed more efficient P450s and higher capacities to detoxify particular plant metabolites produced by their host plants (15). For example, *Papilio polyxenes* having CYP6B1 and CYP6B3, which specialize on *Apiaceae* and *Rutaceae*, demonstrate remarkable efficiency in furanocoumarin detoxification while feeding on their host plants (16). CYP6AB3 in the highly specialized *Depressaria pastinacella* catabolizes imperatorin and myristicin, which are abundant in its target species (17)). Furthermore, the variety of P450s involved in detoxification allows papilionids to thrive on specific host plants(18). P450s that react to phytochemicals may have developed and functionally varied as a result of historical plant adaptation. Allelic P450 variants that have different capacities to detoxify phytochemicals are thought to be induced by *D. pastinacella* to adjust to interspecific differences in furanocoumarins generated by host plants (19). The genome of the western *Apis mellifera* reveals a noticeable drop in the expression of detoxifying genes, indicating an evolutionary history of ingesting relatively chemically innocuous nectar and pollen (20). In contrast, because of their feeding behavior, generalist insects could have a slightly wider variation in P450s than specialist insects because they encounter different classes of plant metabolites in diverse plant species. Furthermore, generalist insects have P450s that are extensively inducible, implying that just a subset of detoxification genes may be active for a given phytochemical (21). The induction of CYP6B8 in Helicoverpa zea, for example, can metabolize flavones, xanthotoxin, quercetin, and a range of other secondary metabolites (22). H. zea also uses CYP321A1 to metabolize phytochemicals comparable to CYP6B8, indicating modifications in phytochemical detoxification mechanisms (23). On the other hand, the capacity to detoxify harmful substances is multifaceted and not restricted to the action of a specific P450 detoxification enzyme. Multiple up-regulated P450s are frequently responsible for increased phytochemical metabolic detoxification and are involved in insect adaptability to host plant defense (24). Numerous P450 genes in the 6B, 321A, and 9A subfamilies of the fall armyworm, *Spodoptera frugiperda*, are sensitive to phytochemicals and implicated in detoxification (25). The expanded P450 subfamilies have been shown to be responsible for phytochemical

detoxification in some generalists, such as the CYP6AE genes in the *Helicoverpa armigera* (26), the CYP6AS genes in the *Apis mellifera* (27), and the CYP6BQ genes in the *Tribolium castaneum* (29).



ECONOMIC IMPORTANCE OF P450

Insecticide resistance and P450s:

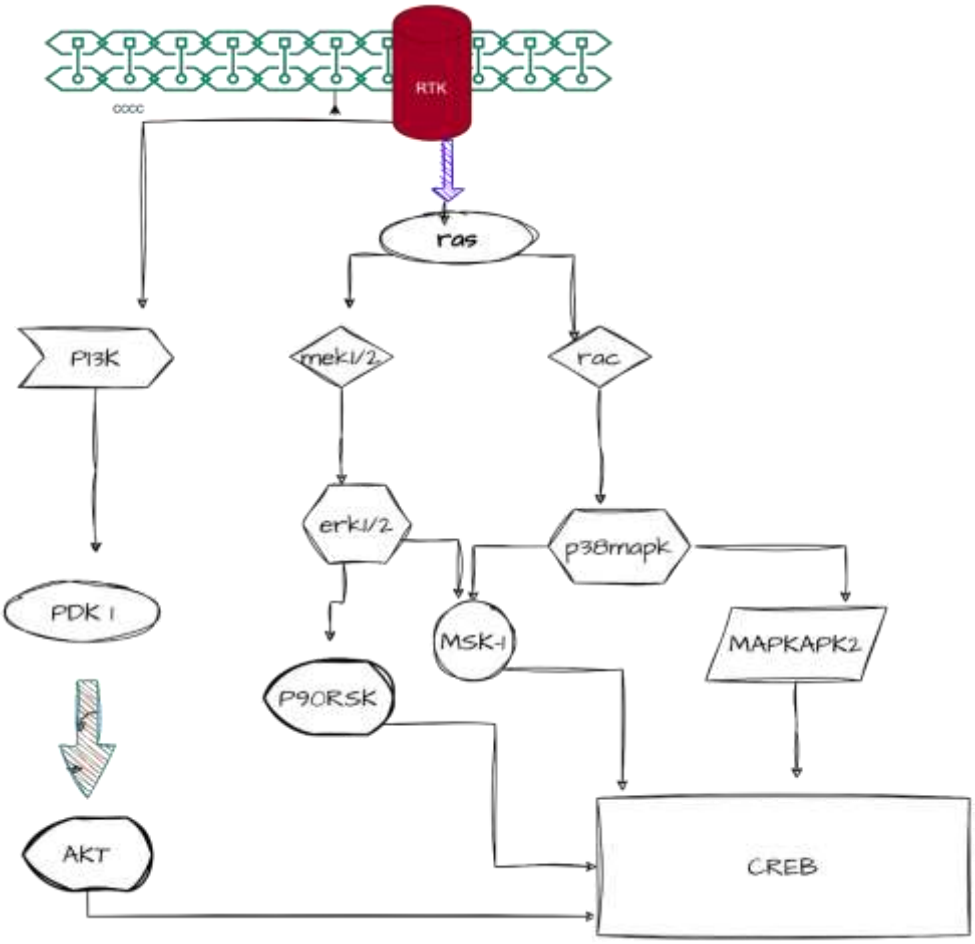
Insecticide resistance has primarily evolved through increased metabolic detoxification, decreased target site sensitivity, altered penetration, and accelerated excretion (30). Insects use resistance mechanisms to phytochemicals and insecticides that are remarkably similar. Insecticides alter the expression of certain groups of insect detoxification genes that are stimulated in host plants by phytochemicals (31). The principal detoxifying enzymes linked with insect resistance to a wide range of synthetic pesticides are likely P450s (32). Many P450s involved in phytochemical detoxification are pesticide-sensitive (33). When insects are exposed to insecticides, certain P450 genes will be co-upregulated. In insecticide-resistant insect strains, constitutive overexpression and induction of these P450 genes is common (34). A considerable number of insect P450s involved in pesticide resistance have been discovered and functionally investigated thus far, with many belonging to the CYP6 family. For example, CYP6ER1, CYP6AY1, and CYP6CW1 are overexpressed in imidacloprid-resistant *Nilaparvata lugens* strains and have been demonstrated to contribute to imidacloprid resistance development (35). CYP6P3 and CYP6M2, involved in detoxifying deltamethrin and permethrin, are highly expressed in all pyrethroid-resistant populations of the *Anopheles gambiae* (36). Furthermore, the role of P450s from other families in pesticide resistance has been well described. Some researchers (37,38) examined the expression, regulation, and significance of insect P450s in pesticide resistance.

Mechanism of P450s and xenobiotic resistance:

The resistance process to synthetic pesticides and phytochemicals is similar, implying that interactions between these toxic compounds and P450-mediated cross-tolerance/resistance may occur (39). Because many synthetic pesticides are chemically similar to plant secondary metabolites, it has been postulated that P450s implicated in insecticide resistance originated from those engaged in phytochemical detoxification (40). The induction of P450s by phytochemicals often enhances the metabolism of other xenobiotics. Thus, prior exposure to more abundant phytochemicals in host plants has been found to condition insect herbivores to withstand insecticides. For example, in *Helicoverpa zea*, induction P450s with xanthotoxin contributes to increased resistance to alpha-cypermethrin (41). Pre-exposure of the essential phytochemical from cotton, gossypol given to *Heliocoverpa armigera* larvae leads to an increase in deltamethrin tolerance(42). Microarray study has demonstrated that gossypol and deltamethrin both stimulate detoxification genes, with distinct P450s upregulated by these two xenobiotics (43).

Similarly, exposure to quercetin enhances lambda-cyhalothrin tolerance, which is related to quercetin activation of CYP6B6, CYP6B8, and CYP321A1 (Chen et al., 2018). Because P450s are inducible by a wide range of xenobiotics, cross-resistance/tolerance between insecticides and environmental contaminants is common. This significantly impacts environmental preservation, resistance management, and pest control efficacy. If the exposure to specific pollutants can increase insect tolerance to insecticides via P450 cross-induction, additional precautions must be taken in areas polluted by such substances. For example, exposure of *Aedes aegypti* larvae to several pollutants, such as the herbicides atrazine, fluoranthene, and glyphosate, and the heavy metal such as copper show greater pesticide tolerance (44;45). DNA microarray study demonstrated P450 cross-induction, showing that environmental contaminants influence the regulation of P450-mediated pesticide response (46). Laboratory investigations have recently revealed that insect adaptation to heavy metal contamination leads to enhanced resistance to insecticides and phytochemicals. Pre-exposure to the heavy metal lead improves larval resistance to a-cypermethrin in the *Spodoptera litura* via inducing CYP6B47 and CYP9A39 (47).
Similarly, heavy metal copper pre-exposure improves *S. litura* larval resistance to b-cypermethrin via activation of CYP6AB12 (48). Elevated expression of CYP6B50 was reported to be required for greater resistance to allelochemical xanthotoxin in copper-treated *S. litura* larvae, implying that environmental contaminants may also modify phytochemical detoxifying capabilities in herbivorous insects (48). The aryl hydrocarbon receptor (AhR), an intracellular nuclear receptor, has been demonstrated to have a role in heavy metals' cross-induction of P450 genes.

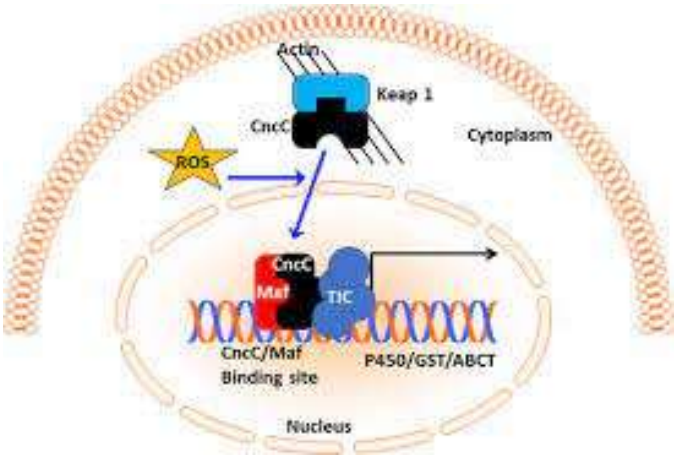
RTK and MAPK signaling:
While much is known about the detoxification mechanisms that confer pesticide adaptation, substantially less is known about the regulatory routes that mediate insect P450 expression. One research linked RTK and MAPK signaling to P450 regulation. A mechanism of action of CYP6CM1 constitutive overexpression in imidacloprid-resistant *Bemisia tabaci*(Yang et al., 2020) has indirectly implicated the RTK/MAPK pathway by showing constitutive overexpression of CYP6CMI that is linked with constitutive overexpression of CREB and its upstream MAPK kinase component P38, as well as constitutive over phosphorylation of CREB and its two upstream MAPK kinase components ERK and p38. Yang et al. (52) discovered that the bZIP transcription factor CREB regulates the overexpression of CYP6CM1, which gives resistance to the neonicotinoid imidacloprid in the whitefly, *B. tabaci*. They also found that imidacloprid activates the MAPK signaling pathway, which phosphorylates CREB in the nucleus. When CREB is phosphorylated, it binds to CRE-like sites in the promoter of CYP6CM1 and stimulates its expression. The trans-regulatory route linked to the over expression of a major P450 detoxification gene in *B. tabaci* was discovered, and the MAPK signaling system and the transcription factor CREB were implicated in pesticide adaptation. According to Sasabe et al (49), at least two xanthotoxin-inducible P450s from substantially diverse subfamilies (CYP6B and CYP321A) contribute to *H. zea* larval resistance to poisonous furanocoumarins and pesticides. According to genomic PCR research, the CYP321A1 gene developed independently of the CYP6B genes found in this insect. Recent research in numerous insect species has found some transcription like the basic leucine zipper (bZIP), basic-helix-loop-helix/Per-ARNT-Sim, and nuclear receptor superfamilies regulate P450-mediated xenobiotic detoxification (50). *Drosophila melanogaster* nuclear receptors such as DHR96 have also been demonstrated to be linked with the control and regulation of xenobiotic resistance by influencing P450 expression (51). Induction of CYP6CM1, which confers imidacloprid resistance in the whitefly *Bemisia tabaci*, is trans-regulated by a bZIP transcription factor, cAMP-response element binding protein (CREB), which is activated by mitogen-activated protein kinase (MAPK) via phosphorylation in response to imidacloprid (52). However, how insects perceive the pesticide signal and activate the MAPK signaling pathway is unclear. Several investigations have highlighted the roles of G-protein-coupled receptors (GPCRs) in extracellular messenger recognition, signal transduction, and MAPK signaling pathway activation (53). GPCRs may operate as putative upstream activators of the MAPK signaling pathway implicated in pesticide resistance due to their participation in controlling P450-mediated insecticide resistance (52). Many investigations in *Drosophila melanogaster* and other insect species have shown that the bZIP factor Cap 'n' collar isoform C (CncC), along with specific heterodimer partner, tiny muscular aponeurosis fibromatosis (Maf), are the major transcriptional regulators of toxins detoxification P450s (51). Transcription factors that initiate P450 gene expression must be continuously over-expressed, and a study revealed that in *S. litura*, reactive oxygen species (ROS) might act as inducers to activate CncC and Maf, which are responsible for P450 gene induction in response to insecticides (53).



RTK/MAP KINASE PATHWAY

The ROS/CncC signaling:

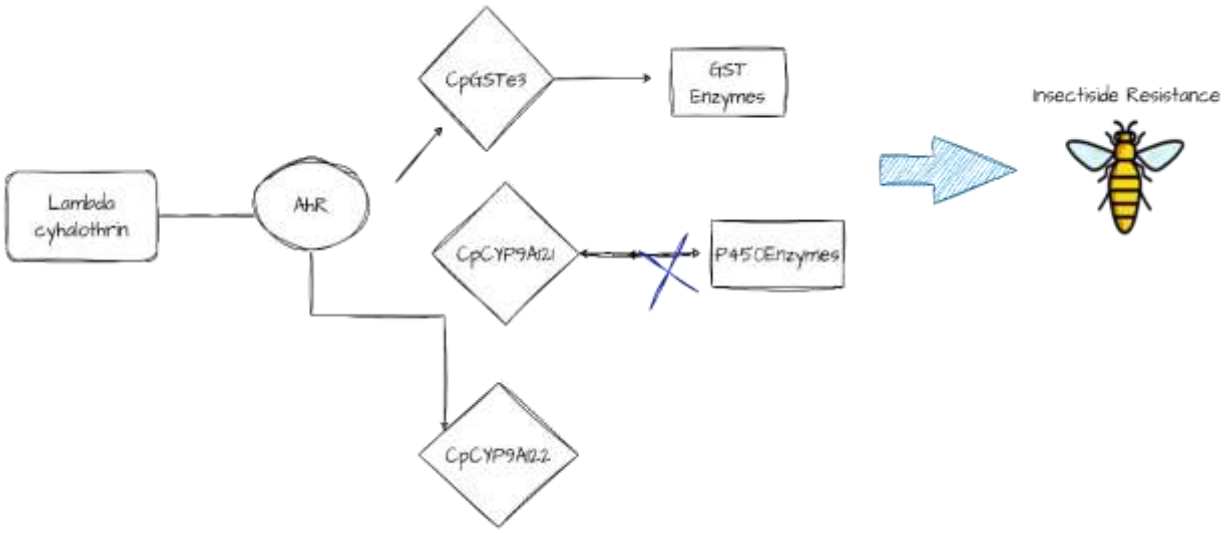
The ROS/CncC signaling pathway is implicated in six biochemical investigations of P450-mediated pesticide resistance (51) and three non-phytochemical inductions of P450s (54). Phenobarbital induction of CYP6A2, CYP6A8, and CYP12D1 in *D. melanogaster* was dependent on both CncC and its 15-bp binding site (55). However, neither of these studies addressed whether phenobarbital acts as a ROS elicitor or an electrophile to induce dissociation of the CncC/Keap1/Cul3 complex and nuclear translocation of CncC. The need for the 15-bp CncC binding site in caffeine induction of CYP6A2 (56) shows that caffeine induction of CYP6A2 is likewise regulated, at least partially, by the CncC/Keap1 pathway. In *Spodoptera litura*, on the other hand, l-cyhalothrin induced a dose-dependent ROS burst as well as the overexpression of CYP6AB12, CncC, and Maf (57).



THE ROS/CncC PATHWAY

AhR/ARNT pathway:

The occurrence of the AhR/ARNT pathway in phytoconstituents regulation of P450s has been interpreted from studies of xanthotoxin induction of CYP6B1 in *Papilio polyxenes* (58) and CYP6B4 in *Papilio glaucus* (59), constitutive co-over expression of AhR, ARNT, and CYP6CY3 have been reported in the nicotine-tolerant *Myzus persicae* nicotian While the two aphid case studies revealed that the AhR/ARNT pathway modulated CYP6CY3 or CYP6DA2 expression (60), more research is needed to determine whether AhR and ARNT regulate enobiotic induction of the two P450 genes, as well as whether AhR binds nicotine or spirotetramat. The induction of CYP6B1 and CYP6B4 by xanthotoxin depended on an overlapping EcRE/ARE/XRE-xan element, and to a lesser extent, XRE-AhR (61). Heterologous co-expression of AhR (Spineless) and ARNT (Tango) homologues in *D. melanogaster* confirmed that the AhR/ARNT system controls both basal and xanthotoxin-stimulated CYP6B1 expression (Insects contain 19-22 NRs, depending on lineage, but exogenous ligands, whether phytochemicals or other xenobiotics are mostly unknown (61). Three insect NRs have been involved in the phytochemical regulation of P450s, including Hnf4 (Hepatocyte nuclear factor 4), HR96 (hormone receptor-like in 96), and Ftz-f1 (Ftz transcription factor 1). HR96 and PXR (pregnane X receptor) (61) are constrained to the nucleus, bind to sterols, and regulate phenobarbital induction of the house fly P450 CYP6D1 in *Drosophila melanogaster* S2 cells (62). The mutation that can lead to the Loss-of-function of HR96, on the other hand, did not lower the inducibility of all 29 phenobarbital-upregulated *Drosophila melanogaster* P450 genes, including CYP6G1, CYP12A4, CYP12D1, CYP6A2, CYP6A8, and CYP6A21 but did release all three phenobarbital-downregulated P450s (CYP309A2, CYP4S3, CY P6A14) from repression by phenobarbital (63). The probable dominance of the ROS/CncC/Keap1 route over the HR96 pathway may explain the failure of the HR96 null mutant to inhibit 29 phenobarbital-inducible P450s (64)



AhR/ARNT PATHWAY

Conclusion

In conclusion, the cytochrome P450 enzyme family plays a critical role in the metabolism of various endogenous and exogenous compounds in insects. The regulation of these enzymes is complex and involves multiple pathways, including transcriptional regulation, post-transcriptional modification, and modulation by environmental factors. The whole specific regulatory route of P450 related detoxification involved in insect adaptation to xenobiotics, however, is still mostly unknown. The involvement of transcription factors in xenobiotic reactions, as well as the molecular mechanisms of P450-mediated detoxification, should be the focus of future research. Understanding the regulatory pathways that control cytochrome P450 enzymes in insects is important for developing new strategies to control insect pests, including the development of new insecticides that can target these enzymes specifically. It also highlights the importance of considering the environmental context in which these regulatory pathways operate, as changes in environmental conditions can have a significant impact on the expression and activity of these enzymes.

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