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The Role of *Anopheles gambiae* P450 Cytochrome in Insecticide Resistance and Infection

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1. Introduction

Anopheles gambiae is the major vector of malaria transmission in sub-Saharan Africa where the disease is responsible for the highest morbidity and mortality worldwide. Malaria, nowadays, is still a major burden causing the death of nearly one million people each year, mostly children under the age of five, and affecting those living in the poorest countries (World Health Organization [WHO], 2010).

Currently, the major obstacles to malaria eradication are the absence of a protective vaccine, the spread of parasite resistance to anti-malarial drugs and the mosquito resistance to insecticides. Controlling mosquito vectors is fundamental to reduce mosquito-borne diseases. In fact, it has been one of the most used and effective method to prevent malaria, namely trough insecticides spraying and impregnated bed nets. These methods are highly dependent on a single class of insecticides, the pyrethroids, which are the most frequently used compounds for indoor residual spraying, and the only insecticide class used for insecticide treated nets (WHO, 2010). The extensive use of a single class of insecticides further increases the risk of mosquitoes developing resistance, which could rapidly lead to a major public health problem mainly in sub-Saharan countries where insecticidal vector control is being used widely (WHO, 2010). Strategies to control malaria are still not enough to totally eliminate malaria transmission, having yet to overcome several difficulties as the development of parasite drug resistance and mosquito-vector insecticide resistance (Yassine & Osta, 2010). Unfortunately the emergence of mosquito populations capable of withstanding insecticide exposure is threatening the efficiency of these control measures.

2. Insecticide resistance

Resistance has been defined as 'the inherited ability of a strain of some organisms to survive doses of a toxicant that would kill the majority of individuals in a normal population of the same species' (Scott, 1999). The evolution of insecticide-resistant mosquito strains is an increasing problem and one of the major obstacles for the control of medical and agricultural arthropod pests. Therefore, a better understanding of its genetic and biological basis is critical. Insecticide resistance can also lead to outbreaks of human diseases when

vectors cannot be controlled. Hence, the elucidation of resistance mechanisms is extremely important for the development of tools to monitor resistance in populations, thereby contributing to mosquito control programs. Although the mechanisms by which insecticides become less effective are similar across all vector taxa, each resistance problem is potentially unique and may involve a complex pattern of resistance *foci* (Brogdon & McAllister, 1998).
The main forms of resistance mechanisms can be divided in two groups: target site resistance, which occurs when the insecticide no longer binds to its target, and metabolic resistance, which occurs when enhanced levels of modified activities of detoxification enzymes prevent the insecticide from reaching its site of action. Alone or in combination these mechanisms confer resistance, sometimes at high levels, to all classes of insecticides.

2.1 Target site resistance

Target site resistance is based on alterations of amino acids in the site of action where the insecticide is supposed to bind, causing the insecticide to be less effective or ineffective at all. Knock down resistance (*Kdr*) occurs due to a single or multiple substitutions in the sodium channel (Martinez-Torres et al., 1998; Ranson et al., 2000a); and alteration in acetylcholinesterase results in decreased sensitivity to insecticides (Mutero et al., 1994). Insecticide resistance has been reported from many insects including *A. gambiae* that showed the presence of insensitive acetylcholinesterase in two different populations that were resistant to carbosulfan, a carbamate insecticide (N'Guessan et al., 2003). Mutations at a single codon in the *Rdl* (resistance to dieldrin) gene have been documented in all dieldrin-resistant insects, and confer both insensitivity to the insecticide and a decrease rate of desensitisation (ffrench-Constant et al., 1998). However, in *A. gambiae* this type of resistance mechanism has not been described so far. Those are examples of target site resistance that is not the object of the present review.

2.2 Metabolic resistance

Metabolic resistance usually involves over-expression of enzymes capable of detoxifying insecticides or modifications in the amino acid sequences that cause alterations in the levels and activity of detoxifying proteins. There are three major enzyme families involved in this type of resistance, glutathione-S-transferases (GST), carboxylesterases and P450 cytochromes. Carboxylesterases are mainly involved in organophosphate and carbamate and to a lesser extent in pyrethroid resistance, while P450 cytochromes are mainly involved in the metabolism of pyrethoids and to a lesser extent, detoxification of organophosphates and carbamates (Hemingway & Ranson, 2000). Glutathione S-transferases are involved in the detoxification of a wide range of xenobiotics, including the organochloride insecticide DDT (Enayati et al., 2005). In *A. gambiae* metabolic resistance to insecticides can be conferred by elevation in the activity of these three classes of detoxifying enzymes.
The over-expression of carboxylesterases as an evolutionary response to organophosphorus and carbamate insecticide selection pressure has been reported in several insects, including mosquitoes (Newcomb et al., 1997; Vulule et al., 1999; Zhu et al., 1999). Organophosphorus and carbamate inhibit B esterases by rapid esterification of the serine residue in the active site, usually followed by a slow hydrolysis of the new ester bond. Therefore, these insecticides can be considered as inhibitors of esterases, because they are poor substrates which have a high affinity for these enzymes (Hemingway & Karunaratne, 1998). Carboxylesterases in large amounts causes resistance as the insecticides are rapidly sequestered, even before reaching the target-site acetylcholinesterase (Hemingway &

Karunaratne, 1998). There are many reports of over expression of carboxylesterases in insecticide resistant mosquitoes including *A. gambiae*, where enhanced production of carboxylesterases was observed in permethrin-resistant mosquitoes (Vulule et al., 1999).
Glutathione S-transferases are a major class of detoxification enzymes that possess a wide range of substrates specificities (Enayati et al., 2005). Elevated GST activity has been implicated in resistance to several classes of insecticides (Ranson et al., 2001). Higher enzyme activity is usually due to an increase in the amount of one or more enzymes, either as a result of gene amplification or more commonly through increases in transcriptional rate, rather than qualitative changes in individual enzymes (Hemingway et al., 2004). The primary function of GSTs is the detoxification of both endogenous and xenobiotic compounds either directly or by catalysing the secondary metabolism of a vast array of compounds oxidised by P450 cytochromes (Wilce & Parker, 1994). GST enzymes metabolise insecticides by facilitating their reductive dehydrochlorination or by conjugation reactions with reduced glutathione to produce water soluble metabolites that are more readily excreted (Wilce & Parker, 1994). They also contribute to the removal of toxic oxygen free radical species produced through the action of pesticides (Enayati et al., 2005). In *A. gambiae* elevated GST levels were shown to be associated with DDT resistance (Ranson et al., 2001). Furthermore genetic mapping of the major *loci* conferring DDT resistance in *A. gambiae* implicate both *cis*- and *trans*-acting factors in the overexpression of GSTs (Ranson et al., 2000b). GSTs in *A. gambiae* were over expressed in a DDT-resistant strain, but only one *GSTE2-2* was able to metabolise DDT (Ortelli et al., 2003).
P450 cytochromes are a complex family of enzymes that are involved in the metabolism of xenobiotics and have a role in the endogenous metabolism. P450 cytochromes mediated resistance is probably the most frequent type of insecticide resistance. They are involved in the metabolism of virtually all insecticides, leading to activation of the molecule in the case of organophosphorus insecticides, or more generally to detoxification (Scott & Wen, 2001). In most cases where a link between insecticide resistance and elevated P450 activity has been shown, the P450 cytochrome belongs to the *CYP6* family (Nikou et al., 2003; Djouaka et al., 2008; Müller et al., 2007; McLaughlin et al., 2008). Although being difficult the identification of the specific P450 cytochrome associated with resistance, several P450 cytochromes were already isolated from insecticide resistant strains (Dunkov, et al., 1997; Kasai & Scott, 2000; Sabourault et al., 2001).

3. Insect P450 cytochromes

P450 Cytochromes are hemoproteins which act as terminal oxidases in monooxygenase systems. P450 cytochromes, whose name originated on its characteristic absorbance peak at 450 nm that appears when these enzymes are reduced and saturated with carbon-monoxide, constitute one of the oldest and largest super families of enzymes being found in almost all living organisms. In the literature, P450 enzymes are known by several names: cytochromes P450 monooxigenases, mixed functions oxidases, microsomal oxidases and heme thiolate proteins.
Insect P450s play a critical role in the metabolism of a wide variety of endogenous and exogenous compounds such as steroids, fatty acids and a wide range of xenobiotics and have also been implicated in vital processes like growth, development, feeding, reproduction, insecticide resistance and tolerance to plant toxins (Feyereisen, 1999; Scott et al., 1998; Scott, 1999). P450 cytochromes are also intimately involved in the synthesis and

degradation of insect hormones and pheromones, including 20-hydroxyecdysone and juvenile hormone (Feyereisen, 1999).

3.1 Nomenclature

To distinguish one of these cytochromes among all the P450s, a standardized nomenclature system was implemented (Nebert et al., 1991; Nelson et al., 1996). Each P450 is named with CYP, followed by an Arabical number for the gene family, a letter for the sub-family and another Arabical number for the gene. Cytochromes P450s with share more then 40% of the amino acids are usually grouped into the same family and members with >55% of the amino acids identical are normally grouped in the same sub-family. However, there are exceptions to these rules (Nelson et al., 1996). As it is based on amino acid similarities, no information regarding the function of each P450 should be assumed from its name.

3.2 Structure

P450s can be divided into classes depending on how electrons from NAD(P)H are delivered to the catalytic site. Class I P450s are found in eukaryotes and are associated with mitochondrial membranes. This class of enzymes requires both a FAD-containing reductase and an iron sulphur redoxin, and catalyzes several steps in the biosynthesis of steroid. Class II enzymes are the most common in eukaryotes and are found in the endoplasmic reticulum. These enzymes only require an FAD/FMN-containing P450 reductase for transfer of electrons. Their functions are extremely diverse and, in eukaryotes, include aspects of the biosynthesis and catabolism of signalling molecules and steroid hormones (Feyereisen, 1999). Class III enzymes are self-sufficient and require no electron donor. They are involved in the synthesis of signalling molecules. Finally, class IV enzymes receive electrons directly from NAD(P)H. Class I and II P450s from all organisms participate in the detoxification or sometimes the activation of xenobiotics and class III and IV enzymes are considered remains of the ancestral forms of P450s involved in detoxification of damaging activated oxygen species (Werck-Reichhart & Feyereisen, 2000).

Most P450s are approximately 500 amino acids long. The core of these proteins is formed by a four-helix bundle, two sets of β sheets, two helices and a coil called the "meander". A characteristic consensus sequence known as the P450 "signature" FXXGXXXCXG, located on the C-terminus of the heme binding region, contains a conserved cysteine that serves as a fifth ligand to the heme iron. There are two other conserved motifs specific of the P450 proteins. One is the DGXXT domain, which corresponds to the proton transfer groove on the distal site of the heme. Another is the EXXR domain, which is probably needed to stabilize the core structure located on the proximal side of heme (Werck-Reichhart & Feyereisen, 2000).

3.3 Microssomal / mitochondrial

In insects both mitochondrial and microssomal P450 systems have been described. The majority of P450 in insects are microssomal, located in the endoplasmic reticulum, and require the flavoprotein NADPH cytochrome P450 reductase as the main electron donor; however cytochrome b_5 is sometimes needed, depending of the substrate and of the P450 cytochrome involved. Mitochondrial P450 are also present, but, differently from microssomal P450, require ferridoxin and a NADPH ferridoxin reductase as electron donor (Scott & Wen, 2001).

3.4 Characterization / function

Cytochromes P450 enzymes catalyse thousands of different reactions, which are based on the activation of molecular oxygen, with insertion of one of its atoms into the substrate, and reduction of the other to form water (Guengerich, 1991). P450s use electrons from NAD(P)H to catalyse the activation of molecular oxygen, leading to the regiospecific and stereospecific oxidative attack of structurally diverse chemicals (Werck-Reichhart & Feyereisen, 2000).

The interaction that occurs between P450 cytochromes and the NADPH–cytochrome P450 reductase is better expressed as a cyclic reaction (Guengerich, 1991) as it is depicted in Figure 1.

The cycle is initiated by the binding of the substrate to the ferric form of the enzyme to form an enzyme-substrate complex, followed by a reduction of the ferric complex by an electron transferred from NADPH via NADPH-cytochrome P450 reductase. Next, the binding of molecular oxygen to the reduced complex forms an enzyme-oxygen-substrate complex followed by the transference of a second electron from NADPH via NADPH-cytochrome P450 reductase or from cytochrome b_5. A second proton is added, which results in the breaking of the oxygen-oxygen bond, releasing one atom of oxygen as water. The oxygen atom remaining is transferred to the substrate, originating an oxidized product, which is released, and a ferric form of the enzyme is once more generated. Then the cycle is re-initiated (Guengerich, 1991).

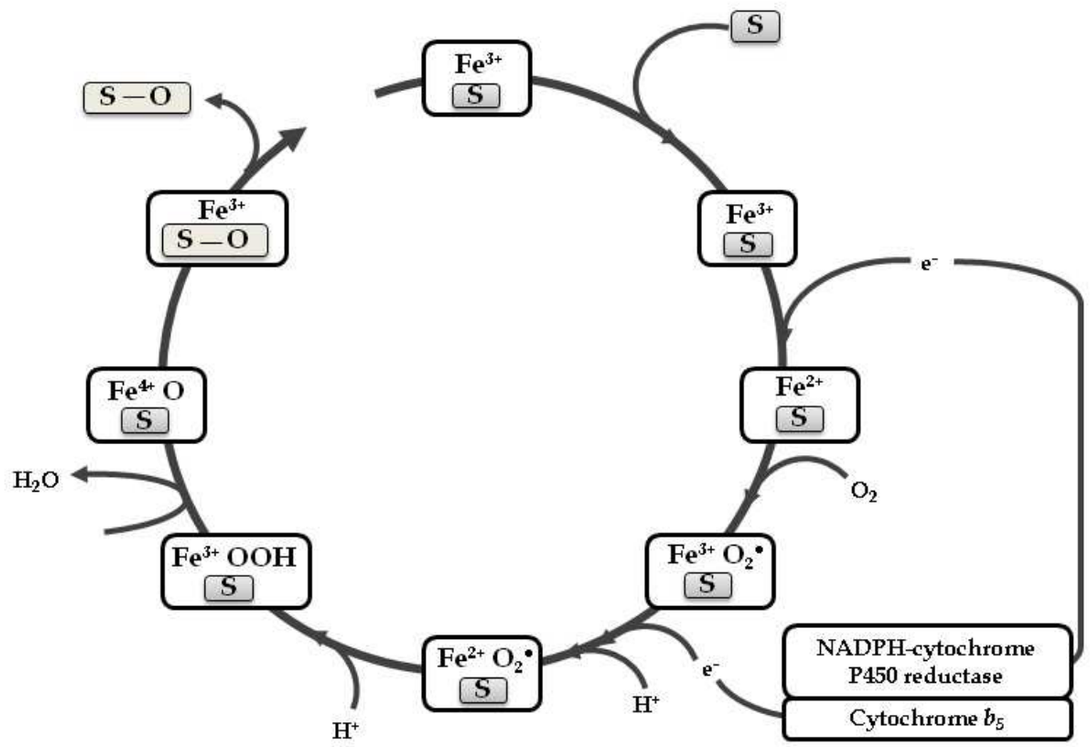

Fig. 1. Catalytic mechanism of P450 enzymes, where S is the substrate.

3.5 Diversity and specificity

The huge diversity of P450 cytochromes is probably due to an extensive process of gene duplication and cases of gene amplification, conversion, genome duplication, gene loss and lateral transfer (Werck-Reichhart & Feyereisen, 2000). Due to their extremely diverse functions, they can be found with different patterns of expression in all types of tissues and in almost all types of organisms. Although being expressed in a wide range of tissues, insect P450s have their highest activity associated with midgut, fat body and malpighian tubules (Feyereisen, 1999; Scott, 1999).

Additionally, P450s metabolise a large number of substrates, probably due to the existence of numerous P450 isoforms and to the broad specificity of some isoforms (Scott & Wen, 2001). Nevertheless the substrate specificity and type of reaction catalysed by each P450 cytochrome is still not well understood.

Their diversity enables individual P450 cytochromes to display different expression patterns related to life stages, tissues, inducers/inhibitors and substrates. There are P450s that are expressed in all life stages (*CYP12* genes) while others are only expressed in adults (*CYP6Z1*) or in larval stages (*CYP6Z3*) (Nikou et al., 2003). Although being found expressed in almost all types of tissues, there are P450s which are tissue specific, while others are expressed everywhere (Feyereisen, 1999; Scott et al., 1998; Scott & Wen, 2001). Expression of P450 cytochromes may also be sex specific, as some P450s showed higher levels of expression in males compared with females (Muller et al., 2007; Nikou et al., 2003).

A large variation in substrate specificity can also be found among different P450s, some being capable of metabolising several substrates while others have only one known substrate (Scott, 1999; Scott et al., 1998). There can be also some overlapping substrate specificity among P450 cytochromes, so that one compound could be metabolised by several enzymes. The production of one or several metabolites from a single substrate also differs depending on the P450s. P450s show a vast variation in response to inducers and inhibitors, each P450 can be induced/inhibited by one or several compounds. Some P450s can also remain unaltered while others are induced or repressed (Scott et al., 1998).

4. *Anopheles gambiae* P450 cytochromes and insecticide resistance

The *A. gambiae* genome has 111 annotated P450 cytochromes (Ranson et al., 2002). The great interest in these cytochromes derives from their role in the oxidative metabolism of insecticides, but only in few cases a definitive link between an increased expression of a specific P450 cytochrome and increased insecticide metabolism has been established.

Increasing reports of specific *A. gambiae* P450 cytochromes being involved in insecticide resistance have been published in the past. The involvement of P450s in pyrethroid resistance started to be demonstrated in *A. gambiae* from Kenyan villages, in synergistic studies using specific P450 cytochrome inhibitors and also given the detection of increased heme levels in resistant mosquitoes (Vulule et al., 1999).

In 2003, Nikou et al., verified that a P450 cytochrome (*CYP6Z1*) was over-expressed in a pyrethroid-resistant strain of *A. gambiae,* and the development of her work pointed to an implication of the involvement of this P450 in conferring pyrethroid resistance to this mosquito (Nikou et al., 2003).

Later, a microarray *chip* was constructed containing fragments from 230 genes associated with detoxification (David et al., 2005) to further study the metabolic based insecticide resistance in *A. gambiae*. From this work resulted the identification of, among other genes,

several P450 cytochromes that were highly expressed in the *A. gambiae* permethrin or DDT-resistant strains (David et al., 2005). Of notice is the P450 cytochrome *CYP325A3,* which belongs to a class that was not associated with insecticide resistance before and which was highly over-expressed in an *A. gambiae* permethrin resistant strain. Additionally, *CYP325A3* was later reported as constitutively over-expressed in a Nigerian pyrethroid resistant strain of *A. gambiae* (Awolola et al., 2009).

In 2007, studies regarding a recently colonised strain of *A. gambiae* from Ghana identified genes whose expression levels were associated with pyrethroid resistance. Among these were three P450 cytochromes (*CYP6M2, CYP6Z2* and *CYP6Z3*) (Muller et al., 2007). These results, together with their location within a cluster of P450 cytochromes in the right arm of chromosome 3 (3R), which is in close association with a pyrethroid resistance QTL (Ranson et al., 2004), strongly support their involvement in insecticide resistance. A subsequent study showed that *CYP6Z2* displays broad substrate specificity, which may be associated with xenobiotics metabolism and detoxification (Mclaughlin et al., 2008). Despite, *CYP6Z2* being able to bind to permethrin and cypermethrin, *CYP6Z2* does not metabolise neither one of these insecticides (Mclaughlin et al., 2008).

In 2008, Djouaka et al. also identified several P450 cytochromes over-expressed in one or more pyrethroid resistant populations of *A. gambiae.* Among these were *CYP6P3* and once again *CYP6M2*. Both genes showed high levels of over-expression in all the resistant populations, but the first was the gene that showed greatest differences. In the same year, *CYP6P3* was also identified as being up-regulated in another highly permethrin resistant *A. gambiae* population (Müller et al., 2008).

Recent studies on *A. gambiae* recombinant proteins *CYP6M2* (Stevenson et al., 2011) and *CYP6P3* (Müller et al., 2008) demonstrated that these enzymes could metabolise pyrethroids. Thus, the up regulation of these P450 cytochromes in pyrethroid resistant populations, strongly supports a key role for these genes to confer pyrethroid resistance in *A. gambiae*.

Highly expressed P450s have been also reported in DDT resistant strains of *A. gambiae* (David et al., 2005). *CYP6Z1* and *CYP12F1* were strongly over-expressed together with other genes, suggesting that multiple genes could contribute to the DDT resistance phenotype. The slightly over-expression of the electron donor cytochrome P450 reductase in the DDT resistant strain further supported a P450-based resistance mechanism in *A. gambiae* (David et al., 2005).

As the above P450 cytochromes, *CYP314A1* was also found to be over-expressed in a DDT resistant strain of *A. gambiae* from Kenia (Vontas et al., 2005), suggesting a possible involvement in the insecticide resistance phenotype. Both *CYP6Z1* and *CYP6Z2* were over-expressed in DDT resistant strains of *A. gambiae* (David et al. 2005). Although being very similar, these two cytochromes have predicted substrate cavities dramatically different and *CYP6Z1* was predicted to be the only one capable of metabolizing DDT. Chiu et al. (2008) through biochemical characterisations supported these predictions and identified *CYP6Z1* as the only P450 cytochrome capable of metabolising DDT, demonstrating its potential as a target to reduce *A. gambiae* resistance to DDT (Chiu et al., 2008).

Another evidence of the involvement of P450s in insecticide resistance is the fact that silencing the main electron donor of P450 cytochromes, the cytochrome P450 reductase, by RNAi, greatly increased the susceptibility of *A. gambiae* to permethrin, emphasising the important chemoprotective role of P450 cytochromes in this process (Lycett et al., 2006).

Nevertheless, although P450s have been clearly associated with insecticide resistance, the identification of specific P450 cytochromes responsible for insecticide resistance is still extremely difficult.

5. *Anopheles gambiae* P450 cytochromes and malaria infection

P450 cytochromes have also been implicated in other vital processes as in *A. gambiae* response to bacterial challenge and to parasite invasion, but the real importance and function of these cytochromes in this process is still not well understood.

A genome expression analysis of *A. gambiae* was made to identify which genes responded to injury, bacterial challenge and malaria infection (Dimopoulos et al., 2002). This study identified three P450 cytochromes, one associated with injury, microbial challenge and oxidative stress; the second associated with the response to septic injury which is similar to a bacterial infection *in vivo*; and the third associated with the response to malaria infection and the presence of lipopolysaccharide (Dimopoulos et al., 2002).

The involvement of P450 cytochromes in response to microbial challenge was established when two P450 cytochromes (*CYP4C27* and *CYP306A1*) were differently expressed in the presence of Gram negative (*Salmonella thyphimurium*) or Gram positive (*Staphylococcus aureus*) bacteria (Aguilar et al., 2005). This involvement was even more evident when a study, trying to implicate the mosquito midgut microbiota in the defense against malaria parasites, showed that there were ten P450s differently expressed in response to *Escherichia coli* and *S. aureus* in the *A. gambiae* midgut twelve hours after an uninfected blood meal (Dong et al., 2009). Between the P450 cytochromes differently expressed there were *CYP4H17, CYP6M3, CYP6AG1, CYP9J5*, two of them were mitochondrial cytochromes, *CYP49A1* and *CYP12F4* (Dong et al., 2009).

Regarding the relation between P450 cytochromes and the response to malaria infection, it was partly unveiled for the first time in a study about the midgut epithelial responses to *Plasmodium* invasion (Vlachou et al., 2005). The study revealed that P450 cytochromes were differentially expressed during different phases of the midgut invasion (before invasion, during invasion and after invasion) as well as when they compared *Plasmodium* wild-type infection with *Plasmodium* that were unable to invade the epithelium. (Vlachou et al., 2005). P450s that stood out in this study were *CYP305A1, CYP304B1, CYP6Z1* and *CYP6M4* (Vlachou et al., 2005). The role of P450 cytochromes in the *A. gambiae* response to malaria infection has been reinforced in the last years. Comparing the *A. gambiae* response to two different *Plasmodium* parasites -*P. berghei* and *Plasmodium falciparum* - showed that the mosquito induced slightly different immune responses to each parasite, and that the mosquito was capable of sensing infected blood constituents and mount an immune response, even in the absence of invading ookinetes (Dong et al., 2006). Although there were different responses between the three experimental groups, in all of them there were P450s differentially expressed in the midgut (*CYP6AG1, CYP6M4, CYP6M1, CYP9J5* and *CYP12F3*) and in the fat body (*CYP6AG1* and *CYP4G17*), reinforcing, their involvement in response to malaria infection.

Further evidence of the link between P450 cytochromes and the mosquito's response to malaria infection came from different studies. First, the effect on gene regulation of the presence of chloroquine in an uninfected blood meal and in a *Plasmodium* infected blood meal was investigated (Abrantes et al., 2008). This work showed that chloroquine affects the abundance of transcripts which encode proteins involved in a variety of processes,

including P450 cytochromes that were differently expressed in the *P. berghei* infected blood meal (*CYP9L1, CYP304B1* and *CYP305A1*). A second study focused on the role of *A. gambiae* detoxification enzymes, from the three major families involved in detoxification, GSTs, carboxylesterases and P450 cytochromes, in the response to *Plasmodium* infection (Félix et al., 2010). In this study the impact of *P. berghei* infection was analyzed at two time points: one day following the blood meal, during which parasites invade the midgut epithelium, and eleven days after the blood meal when sporozoites were starting to be released to the hemolymph; in two different tissues, midgut and fat body. At day one after the *Plasmodium* infected blood meal they found 17 P450 cytochromes down-regulated and 5 P450 cytochromes up-regulated, including *CYP9L1, CYP304B1, CYP325H1, CYP6M2* and *CYP6Z2* in the midgut, and 5 P450 up-regulated and 1 down-regulated in the fat body, including *CYP12F2, CYP6M2, CYP6M3* and *CYP4G17*. At eleven days after an infected blood meal they found 2 P450 cytochromes up-regulated and 3 down-regulated in the midgut and 1 P450 cytochrome up-regulated and 1 down-regulated in the fat body. The high number of P450 cytochromes differently expressed by the presence of *P. berghei* parasites in different phases of infection and in different tissues suggests that P450 cytochromes are deeply involved in the mosquito response to *Plasmodium* infection, having an important role in different development stages of the parasite and covering different tissues of the mosquito. More specifically, these P450 cytochromes might have a direct role in *Plasmodium* response during the parasite invasion of the midgut epithelium as this is the moment and tissue where more P450 were differentially expressed. The over expression of these P450 cytochromes could be part of a mosquito response mechanism to parasite invasion occurring in the midgut. One possibility is that P450s are involved in the cytoskeleton rearrangement (Vlachou et al., 2005; Vlachou & Kafatos, 2005), or alternatively P450s could be involved in the production of nitric oxide and other reactive oxygen radicals that are induced by *Plasmodium* invasion of the midgut epithelium (Han et al., 2000; Luckhart et al., 1998). The blood meal *per si* generates metabolic changes that are also expected to increase the oxidative stress in the mosquito midgut, which is augmented by the presence of *Plasmodium* parasites (Molina-Cruz et al., 2008). Moreover, other parasite killing mechanisms also induce oxidative stress inside the host which, although helping to eliminate the parasite, are also toxic to the host cell. The high level of oxidative stress inside the host cell could trigger cellular and molecular regulation of these P450 cytochromes, at this time point, being responsible for host detoxification and parasite elimination.

Mosquito hemocytes mediate important cellular immune responses including phagocythosis, encapsulation and secrete immune factors such as antimicrobial peptides and mediate melanization. Recently, studies were made to characterize the role of *A. gambiae* hemocytes in mosquito immunity, consisting in a genome-wide transcriptomic analysis of adult female hemocytes following infection by bacteria and *Plasmodium* parasites (Baton et al., 2009). This work showed that *CYP325H1* and *CYP6M1* were differently expressed in the presence of *Micrococcus luteus*, a Gram-positive bacteria (Baton et al., 2009), reinforcing the role of P450 cytochromes in response to microbial challenge. This work also showed that *CYP325H1* was differently expressed 24 hours after the infected blood meal, during *P. berghei* ookinete invasion of the midgut epithelium. Moreover, *CYP6AG1* and *CYP6M3* were also differentially expressed 19 days after the infected blood meal, during *P. berghei* sporozoite migration through the hemolymph (Baton et al., 2009), suggesting that P450 cytochromes have a role in the response to malaria infection by hemocytes. Another study aiming to analyze the transcriptional profile of circulating *A. gambiae* hemocytes during *P.*

berghei infection showed that *CYP6Z1*, *CYP6M2*, *CYP6M3* and *CYP12F2* were differently expressed at 24-28 hours after an infective blood meal (Pinto et al., 2009), valuing the importance of P450 cytochromes on the hemocyte response to malaria parasite invasion.

6. Conclusion

The role of P450 cytochromes during *Plasmodium* invasion is still poorly understood, but it may play out to be of utmost importance to combat malaria transmission. Here, we intend to bring an update review on the connection between P450 cytochromes and the *A. gambiae* response to malaria infection, identifying several P450 cytochromes that probably are, directly or indirectly, involved in the response to *Plasmodium* invasion. We have also reviewed the implication of P450 cytochromes in *A. gambiae* insecticide resistance. However, uncovering the objective role of these cytochromes in insecticide resistance, that is naming specific cytochromes and describing in detail the processes in which those specific P450s are involved is still extremely difficult.

The consistent detection of differential expression of P450 cytochromes, in studies about either insecticide resistance or the response to malaria infection, suggests that the role of these P450s could be similar in these two processes. Nevertheless, the real importance and function of P450 cytochromes in these processes is still not well understood neither the possibility of interplay between infection and insecticide resistance. One of the P450 cytochromes with expression altered in response to insecticides and *Plasmodium* infection was *CYP6M2* that, was highly over-expressed in a pyrethroid-resistant strain of *A. gambiae* mosquitoes (Muller et al., 2007) and also highly over-expressed in response to *Plasmodium* infection in both the midgut and the fat body 1 day after an infected blood meal (Félix et al., 2010). These results suggest that the role of *CYP6M2* might be the same in response to insecticides and infection, or that these two processes might share the activation mechanism of *CYP6M2* expression. *CYP6M2* could also function as an endogenous mediator, acting as the first response to different challenges, which would explain being increased by parasite infection and insecticide exposure. Similar to *CYP6M2* is *CYP6Z1*, yet another P450 cytochrome that was over-expressed in insecticides-resistant strains of *A. gambiae* (David et al., 2005; Nikou et al., 2003) and was also over-expressed in response to *Plasmodium* infection (Vlachou et al., 2005). The increase in the expression of this P450 could function as an immediate response to an exogenous challenge or *A. gambiae* could have the same mechanism of response, including over-expression of specific P450 cytochromes, to parasite infection and insecticide exposure. *CYP6Z2* was highly over-expressed in a pyrethroid-resistant strain (Müller et al., 2007), but opposite to *CYP6M2*, was down-regulated in the midgut of *A. gambiae* at day 1 and day 11 after an infected blood meal (Félix et al., 2010). These results suggest a different role for *CYP6Z2* in response to the insecticide and to parasite infection, however, we have to take into account that, although being able to bind to permethrin and cypermethrin, *CYP6Z2* does not metabolise these compounds (Mclaughlin et al., 2008). So the over-expression of *CYP6Z2* in a pyrethroid-resistant strain might be associated with different processes other than insecticide resistance.

A more complete knowledge about the factors involved in P450 cytochromes response to malaria infection and insecticide resistance is extremely needed for the implementation of efficient malaria and vector control programmes, including strategies able to adapt to different types of resistance. Although the interaction of insecticides with P450 enzymes has been studied, many of its aspects still remains poorly understood. Grasping the underlying

processes in this interaction might help mitigate the problem of insecticide resistance, and therefore contribute to the control of malaria and other human diseases.

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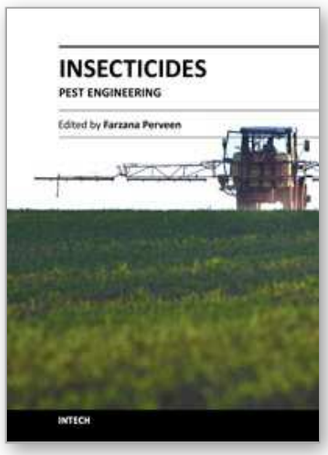

Insecticides - Pest Engineering

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This book is compiled of 24 Chapters divided into 4 Sections. Section A focuses on toxicity of organic and inorganic insecticides, organophosphorus insecticides, toxicity of fenitrothion and permethrin, and dichlorodiphenyltrichloroethane (DDT). Section B is dedicated to vector control using insecticides, biological control of mosquito larvae by Bacillus thuringiensis, metabolism of pyrethroids by mosquito cytochrome P40 susceptibility status of Aedes aegypti, etc. Section C describes bioactive natural products from sapindacea, management of potato pests, flower thrips, mango mealy bug, pear psylla, grapes pests, small fruit production, boll weevil and tsetse fly using insecticides. Section D provides information on insecticide resistance in natural population of malaria vector, role of Anopheles gambiae P450 cytochrome, genetic toxicological profile of carbofuran and pirimicarp carbamic insecticides, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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