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Detoxification Enzyme Systems and Transcriptional Regulation in the Fireflies: A Comprehensive Review

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SUMMARY: *Insects are highly adaptable and efficient in their ability to synthesize and metabolize diverse chemical compounds. The firefly Pyrocoelia spp., (Coleoptera: Lampyridae), is confronted with various chemical assaults throughout its life cycle, the defensive toxins secreted by snails and slugs during firefly larval predation, synthetic pesticides during adult stage, and the risk of auto-toxicity from endogenously produced lucibufagins. Despite the ecological, cultural and economic significance and conservation concerns surrounding fireflies, a clear understanding of their detoxification processes at gene and molecular level is lacking. This review discusses molecular basis of major detoxification enzyme superfamilies, viz., Cytochrome P450 monooxygenases (P450s), Glutathione S-transferases (GSTs), UDP-glycosyltransferases (UGTs), Carboxylesterases (CarEs), and ATP-binding cassette (ABC) transporters. Further the transcriptional regulatory networks, including various pathways that contribute to the induction and expression of these defense genes are discussed in detail. This provides a rationale and a roadmap for developing molecular biomarkers for ecotoxicological risk assessment, deriving conservation strategies for declining firefly populations, and helps in understanding the evolution of chemical adaptation in non-model insects.*

KEYWORDS: *CncC/Keap1 pathway, Cytochrome P450, Detoxification, Ecotoxicology, Firefly, Lucibufagin*

1 The Ecological need for Detoxification

"*Insects are the premier chemists of the world, far ahead of humans in their capacity to synthesize substances* - Thomas Eisner - Father of chemical ecology". The evolution of insects is correlated with the continuous adaptation against diverse chemical challenges [1]. From the secondary metabolites of plants to the defensive secretions of prey and the toxins produced by microbial pathogens, the chemical environment has been a primary selective force shaping insect physiology and genomics [2]. The constant evolutionary pressure of chemical threats has caused insects to develop a larger and more diverse set of genes specifically for detoxifying foreign substances, known as xenobiotics. Similar to most insects, fireflies' entire life cycle is presented with distinct chemical challenges.

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Fireflies *Pyrocoelia* spp., (Coleoptera: Lampyridae) are widely known for their ability to produce light through bioluminescence [3]. Fireflies have an excellent coming out numbering more than 2000 species described, and taking up all the continents but Antarctica. Their habitats are so varied that they can be found in the humid undergrowth of a tropical rainforest in Southeast Asia as well as in the wetlands and grasslands of North America which are temperate. This extensive dispersal has resulted in spectacular niche specialisation with certain species being quite aquatic in their pupal stage and others living in arid conditions. Thus, this great range of geographical distribution subjects various lineages of fireflies to a distinct mosaic of natural and anthropogenic toxins, and various refined and diversified detoxification toolkit is needed to sustain their presence. Along with it, fireflies are of significant cultural significance in the world culture, especially in Asian history [4]. They have been famous in recent decades because of their serious economic returns in the form of ecotourism with well-liked yearly exhibitions in attractions such as the Great Smoky Mountains in the USA, the banks of rivers in Malaysia and the hills of Taiwan.

Besides the direct economic gains, fireflies are also important bioindicators because the abundance and good health directly indicate the integrity of the ecosystem. Fire fly predatory larvae can be considered a natural and biological control, which feeds on snails and slugs among other invertebrates, it is a key player in the control of mollusc populations and hence plant communities and agricultural crops are safeguarded [5]. These extremely polyphagous mollusks eat an enormous variety of plant species, prefer to protect themselves through by secreting a complex mucus that contains unpalatable and possibly toxic glycoproteins, which is a very great biochemical challenge to the firefly predator. These molluskan pests cost the world billions of dollars per year in crop losses and control. In order to mitigate these toxins in the diet, the firefly larva has to have a robust enzymatic defense system in its midgut [6]. Such continuous contact with a certain set of natural toxins has a high potential to put a firm selection pressure on its repertoire of detoxification genes.

After the metamorphosis, the adult firefly must survive in a range of highly polluted ecological niches with anthropogenic pollutants [7]. Synthetic pesticides that are regularly employed in farm pest control could cause a catastrophic outcome on non-target organisms such as fireflies even at concentrations that were not lethal. When exposed continuously to these insecticides fireflies have been found to experience disturbed bioluminescent mating cues, low fecundity, and short lifespan, which reduces the amount of fireflies in the world. Such constant chemical pressures require a powerful detoxification mechanism to survive, the molecular nature of which is under serious under-study [8]. Besides, fireflies also secrete an unpalatable defensive steroid known as lucibufagins that serve as a warning signal for predators. The existence of this potent toxin is a powerful defense that also requires a self-tolerance sequestering mechanism to avoid auto-toxicity.

Among the beetles of the order Coleoptera, a few model pests like the red flour beetle (*Tribolium castaneum*) and the Colorado potato beetle (*Leptinotarsa decemlineata*) are thoroughly genetically characterized [9]. In this aspect, fireflies remain "non-model" organisms where a majority of research was focused on the fascinating biochemistry of their bioluminescence and little is known about their well-required detoxification mechanisms. A bibliometric co-occurrence analysis of firefly-related publications further supports this bias (Figure 1).

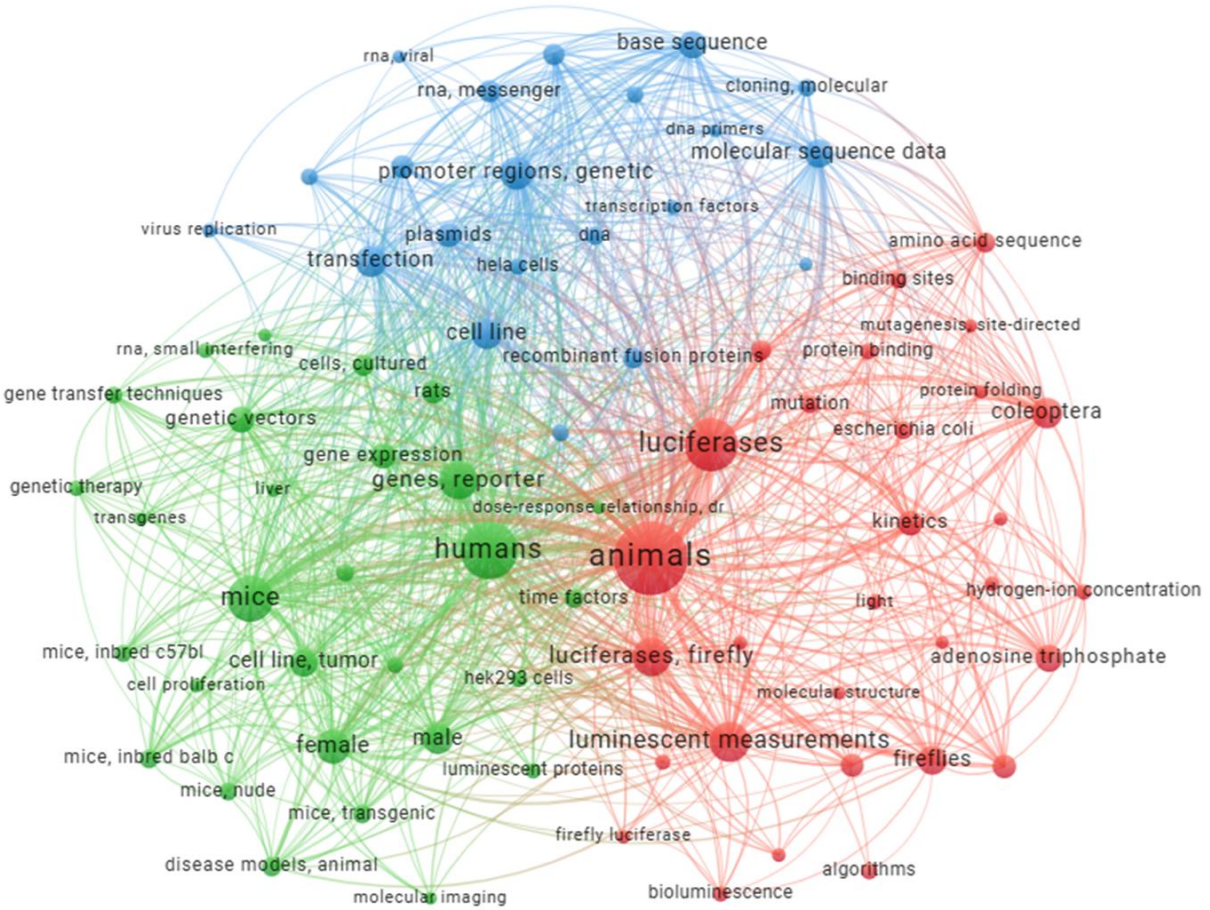

Figure 1: Bibliographic keyword co-occurrence network of firefly-related research from PubMed

In **Figure 1,** Each node represents a keyword; the size of the node reflects its frequency of occurrence, and the thickness of the links indicates the strength of co-occurrence between terms. Colors represent distinct clusters of related research themes: red (bioluminescence, luciferase, coleoptera), green (animal and human model studies), and blue (molecular and genetic mechanisms).

The research landscape is dominated by investigations into the luciferase enzyme (red cluster, "luciferases," "kinetics") and its widespread application as a reporter gene in molecular and biomedical research (green and blue clusters, "genes, reporter," "mice," "cell line," "promoter regions"). In stark contrast, research into the firefly addressing detoxification, xenobiotic metabolism, or ecotoxicology is comparatively sparse. That difference highlights the fact that although the most well-known product of the firefly is a staple of the modern lab, the firefly is an organism with a very poorly studied history, and especially its adaptation to its ability to live in chemically hostile conditions. This knowledge gap can now be filled using next-generation sequencing (NGS) technologies and, specifically transcriptomic analyses to enable deep molecular analysis of any organism, whether it is a model or not [10]. Thus, this review will aim at giving a clear and detailed description of the detoxification in fireflies. Precisely, the current paper will set out to: (i) synthesize the existing molecular knowledge of the main detoxification enzyme superfamilies (P450s, GSTs, UGTs, CarEs and ABC transporters) in Lampyridae; (ii) conceptualize the main transcriptional regulatory pathways, namely CncC/Keap1 and nuclear receptor pathways, which correspond to the regulation of the expression of such defense genes; and (iii) suggest a rationale and a plan of action in

future research.By bridging this knowledge gap, this review seeks to provide a foundation for developing molecular biomarkers for ecotoxicological assessment and advancing our understanding of chemical adaptation in non-model insects.

2 Major Detoxification Gene Superfamilies

The insect detoxification system is a multi-layered network composed primarily of four large and functionally diverse gene superfamilies. These enzymes recognize, modify, and excrete toxic substances in a three-phase coordinated process.

2.1 Phase I: Cytochrome P450 Monooxygenases (CYPs)

The CYPs are a well adaptable and largest multipurpose group of detoxification enzymes. Based on the amino acid sequence identity these CYPs are further classified into clans, families, and subfamilies [11]. The clans CYP3 and CYP4 are highly evolved in insects, and contain a number of genes involved in toxin metabolism [12]. CYPs belonging to other families - CYP6, CYP9, and CYP12 are related to insecticide resistance.

Most of Phase I detoxification is regulated by CYPs. CYPs are globose ring-shaped proteins that have a heme group (iron atom (Fe)) in the center and are membrane-bound on the endoplasmic reticulum. They function as monooxygenases and combine a single molecular atom of oxygen (O_2) with the toxin substrate (R) with the help of the NADPH-cytochrome P450 reductase (CPR) cofactor. This reaction shifts electrons from NADPH to the heme core of the P450 enzyme, which results in a water molecule besides attaching a hydroxyl group (-OH) to the substrate [13] (Figure 2). This adds polarity to lipid soluble toxins, which subsequently reveals a functional group that is addressed by Phase II enzymes. An insect genome has more than 100 CYP genes and one P450 enzyme can metabolize dozens of various compounds.

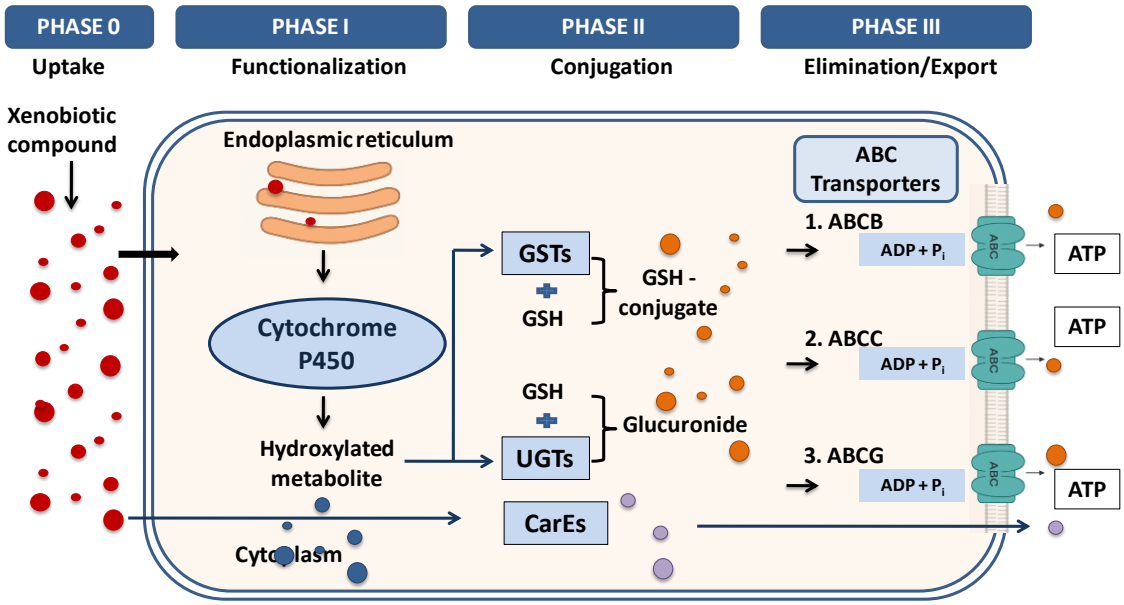

Figure 2: Schematic representation of the xenobiotic detoxification pathway

In **Figure 2,** the detoxification process occurs through four major phases. Phase 0 represents the uptake of xenobiotic compounds into the cell. Phase I (Functionalization) is mediated by Cytochrome P450 monooxygenases (CYPs) located in the endoplasmic

reticulum, which convert lipophilic xenobiotics into more polar hydroxylated metabolites. Phase II (Conjugation) involves enzymes such as glutathione S-transferases (GSTs) and uridine diphosphate glucuronosyltransferases (UGTs), which conjugate the Phase I metabolites with endogenous molecules like glutathione (GSH) or glucuronic acid to form water-soluble conjugates. Phase III (Elimination/Export) is facilitated by ATP-binding cassette (ABC) transporters—ABCB, ABCC, and ABCG—that use ATP hydrolysis to actively efflux conjugated or hydrolyzed metabolites out of the cell. Together, these sequential and coordinated processes enable efficient detoxification and protection against xenobiotic stress. Carboxylesterases (CarEs) act independently by hydrolyzing ester- and amide-containing xenobiotics, often completely detoxifying the parent compound.

CYPs are the primitive enzymes well documented for detoxifying synthetic insecticides such as pyrethroids, organophosphates, and neonicotinoids [14]. Over expression or mutation of a single CYP gene can confer high levels of resistance. CYPs are also critical for herbivorous insects to overcome the chemical defenses of their host plants, such as furanocoumarins, alkaloids, and gossypol. The CYPs enzyme catalyzed reaction model is as follows:

$$R + O_2 + NADPH + H^+ \xrightarrow{CYPs/CPR} ROH + H_2O + NADP^+ \quad (1)$$

This formula describes how CYPs enzymes, with the assistance of NADPH cytochrome P45O reductase (CPR), introduce single-molecule oxygen (O_2) into the substrate (R) to generate hydroxylated metabolites (ROH), while also producing water (H_2O) and regenerating $NADP^+$.

The formula is added in the paragraph describing the function of CYPs enzymes to illustrate how they increase the polarity of lipophilic toxins through oxidative reactions.

The study on firefly CYPs has shown that they have varied and, in many cases, very distinct functions, unlike their more familiar counterparts of insects. The genomic studies accomplished the first on-foundation work in Photinus pyralis which revealed a rich repertoire of 86 candidate CYP genes representing all four major clans and formed the foundation of functional studies. These researches have since associated CYPs with endogenous as well as xenobiotic metabolism. As an example, recent studies have assigned particular CYP families to defensive lucibufagin biosynthesis and the others to the ecdysteroid biosynthesis to molting and hormone regulation. In their classic non-toxicity pathology, the exposures of the pollutant benzo[a]pyrene (BaP) initiates a stronger upregulation of CYP3A, CYP9, and CYP6AS5 genes in Luciola leii. By emphasizing the originality of firefly adaptations it was demonstrated that the "drug metabolism-cytochrome P450" pathway was hyper-stimulated to enable aquatic larvae to withstand the stressor such as hypoxia, an impressive demonstration of the CYPs facilitation of a dramatic ecological shift of the typical terrestrial, herbivorous insect life.This functional plasticity is mirrored at the genomic level by a massive, lineage-specific expansion of the CYP4G subfamily, which contrasts sharply with the pesticide-driven expansions of CYP6 or CYP9 families in agricultural pests. While conserved for cuticular hydrocarbon synthesis in most insects, its dramatic expansion in fireflies suggests its co-option for novel physiological functions, possibly related to the complex biochemistry of the lantern.

2.2 Phase II: Conjugative Enzymes

Phase I substrates are further metabolized by the Phase II conjugating enzymes. These enzymes conjugate the substrates to the readily available highly polar endogenous molecules, making them effectively neutral and excretable.

2.2.1 Glutathione S-transferases (GSTs)

GSTs are a ubiquitous and diverse family of enzymes central to Phase II detoxification. GSTs catalyze the conjugation of the tripeptide glutathione (GSH) to a wide range of electrophilic substrates. This reaction renders the substrate less reactive and far more water-soluble (Figure 2). Insect GSTs are primarily cytosolic and are classified into several classes, with Delta and Epsilon being insect-specific and heavily implicated in detoxification. They are frequently overexpressed in resistant strains. The theta, sigma and zeta classes play more endogenous metabolic roles. They are also the top of the list in reducing oxidative stress, in addition to direct conjugation of xenobiotics. The numerous insecticides and their metabolites are strong oxidants that produce reactive oxygen species (ROS). These ROS can be directly detoxulated by GSTs and other damaged biomolecules can be restored, and thus they form an important part of cellular defense. The calculation model for GSTs catalyzing the binding of glutathione (GSH) to substrates is as follows:

$$R-X+GSH \xrightarrow{GSTs} R-S-G+X^{-} \quad (2)$$

This formula describes how GSTs catalyze the binding of glutathione (GSH) to electrophilic substrates (R-X), generating glutathione complexes (R-S-G) and releasing anions (X^{-}). The company can explain how it connects polar molecules to toxins through conjugation reactions, making them more soluble in water and excreted from the body.

Although the same general enzymatic toolkit appears to be used in fireflies, genomic analysis of Photinus pyralis confirms that the insect has a common insect GST toolkit [15], a more recent study has shown that it performs a function that is highly specialized and divergent due to their unusual chemical ecology. This observation is quite opposite to the conventional detoxification paradigm where GST upregulation is an organism-wide reaction to foreign toxins. Rather it has been powerfully implied that fireflies have recruited this primitive system of detoxification to a vital internal purpose: the self-detoxification of their own powerful chemical weapons.

The specialization that is emphasized under this functional specialization signifies a highly complex evolutionary adjustment. Such theme of enzyme family reuse to create new chemical controls is also reflected in other Coleoptera. As an example, an enzyme-based study of the transcription program of the Malpighian tubules of the giant mealworm (Zophobas morio) identified a novel remodelling-based detoxification mechanism that is interesting because enzymes in the firefly luciferase superfamily initially catalyzed conjugation reactions [16]. Thus, the endogenous defense chemistry in the firefly, in which it uses GSTs, is one of the most interesting examples of how metabolic machineries became adapted to fulfil a distinct ecologic role so that its systems of detoxification are the focus of research to understand how chemical defense and autotoxicity evolved.

2.2.2 UDP-glycosyltransferases (UGTs)

UGTs are another major Phase II family. They catalyze the transfer of a glycosyl group (e.g., glucose) from a UDP-sugar donor to a toxic substrate. Glutathionylation referring to the glycosylation of the substrates increases their polarity and facilitates excretion. UGTs are particularly important for metabolizing plant phenols and other hydroxylated compounds (Figure 2).

About 73 candidate UGT genes were identified in the *P. pyralis* genome. This large repertoire indicates a robust capacity for Phase II detoxification. By adding a sugar moiety, UGTs render toxins highly water-soluble and mark them for removal from the cell by membrane transporters. Similar to specific CYPs and GSTs, a subset of UGT genes was

found to be significantly upregulated in the glands that produce lucibufagin defensive steroids. In the aquatic firefly *Luciola leii*, exposure to the pollutant benzo(a)pyrene (BaP) led to a significant up-regulation of UGTs. In particular, it was always the same UGT gene which was actively induced throughout the time course of exposure to BaP, which suggests that the gene is active in related to Metabolism of the xenobiotic. On the other hand, another UGT gene (UGT2B10L) was found to be down-regulated, which indicates that within this family of enzymes, the response is complex and differs. The transcriptional regulation in fireflies is opposed to the regulatory mechanisms in other species. As an example, a research on the human UGT2A1 enzyme has revealed another level of control in which microRNAs (miR-196a-5p and miR-196b-5p) post-transcriptional regulation of the enzyme demonstrates the heterogeneity of the detoxification evolutionary strategies [17]. The UGTs catalyzed glycosylation transfer process is as follows:

$$R-OH+UDP-G \xrightarrow{UGTs} R-O-G+UDP \tag{3}$$

This formula describes how UGTs enzymes transfer the glycosyl group (G) in uridine diphosphate (UDP-G) to the hydroxylated substrate (R-OH), generating glycosidic complexes (R-O-G) and releasing uridine diphosphate (UDP). This formula can be used to illustrate how glycosylation increases the water solubility of toxins and promotes excretion.

2.3 Phase III: Transporters of ATP-Binding Cassettes (ABCs)

The final process of the detoxification process involves the removal of the metabolized xenobiotics out of the cell. This is the primary role of the Phase III efflux pumps, the most striking of which is ABC transporter. ABC transporters refer to large, membrane-bound proteins that translocation of many different types of substrates across biological membranes is actively powered by the energy of ATP hydrolysis (Figure 2). Some parent xenobiotics and the conjugated metabolites generated by Phase II enzymes are examples of their substrates.

The ABC superfamily is organized into multiple subfamilies (A-H). Insects are most often connected with their xenobiotic transport done by the ABCB, ABCC, and ABCG subfamilies. They are greatly expressed in excretary tissues like in middle intestine and Malpighian tubules. They are the ultimate gatekeepers, and as such, dangerous substances and their metabolites are properly cleared out of the system of the organism. All of these are increasing the significance of ABC transporters in resistance. High expression of certain transporters may lower the concentration of an insecticide in the intracellular sites, and hence the insecticide would fail to reach its target site. This is a very potent mechanism because it may frequently become resistant to various number of insecticide classes, whose structures are not quite identical, example - resistance to Bt toxins in Lepidoptera [18].

P. *pyralis* is demonstrating an extensive repertoire of 64 candidate genes of ABC transporters, and the relevant subfamilies are all well represented, which also speaks to the importance of transport-based biochemistry as an evolutionary investment. They are also envisaged to contribute to the efflux and sequestration of lucibufagins, which helps them store them and release them in the form of defensive secretions. This is a co-option of the detoxification process into which transporters are not only deployed to stop foreign agents but are also deployed to regulate and weaponize native toxins as well. One particular ABC transporter, ABCG8, was discovered to be an A. leii lineage-specific DEG in Aquatica leii, and which participates in freshwater adaptation. Its role in this regard is associated with the ATP production and energy metabolism implying a co-option of energy management during the process of environment adaptation as well as xenobiotic efflux [19]. The ABC transporter utilizes ATP hydrolysis to drive the transport of substances as follows:

$$ATP + H_2O \xrightarrow{\text{ABC transporters}} ADP + P_i + \text{Substrate export} \quad (4)$$

This formula describes how ABC transporters generate adenosine diphosphate (ADP) and inorganic phosphate (P_i) by hydrolyzing ATP, and the released energy is used to drive the transmembrane transport of substrates such as conjugated toxins. This formula can be used to illustrate how it actively transports and clears metabolic waste within cells.

The elimination of the metabolized xenobiotics from the cell is the last stage in the detoxification process. This is the main function of the Phase III efflux pumps, the most notable of which are ABC transporters. ABC transporters are big, membrane-bound proteins that actively move a wide variety of substrates across biological membranes using the energy from ATP hydrolysis. Some parent xenobiotics and the conjugated metabolites generated by Phase II enzymes are examples of their substrates.

2.4 Carboxylesterases (CarEs)

CarEs are the critically important detoxification enzymes irrespective of the phases of detoxification, particularly for certain classes of insecticides. CarEs are hydrolases that cleave ester bonds, a chemical linkage found in organophosphate, carbamate, and pyrethroid insecticides. A single hydrolytic step often suffices to detoxify the parent compound completely (Figure 2). CarEs contribute to insecticide resistance via two primary mechanisms: Upregulated CarEs can rapidly hydrolyze incoming insecticides, preventing them from reaching their nervous system targets. Alternatively, in well-documented cases of resistance, massive overexpression of a CarE gene generates such high protein concentrations that the enzyme acts as a stoichiometric sink, binding and physically sequestering the insecticide at a rate exceeding its hydrolysis.

The midgut transcriptome of *P. pyralis* contains 30-50 CarE genes pertaining to hydrolysis of dietary esters and regulation of endogenous compounds [20]. In Sclerotia aquatilis, about 56 transcripts governing Juvenile Hormone Esterase (JHE), a key CarE, were identified. JHE expression is highest during the larval stage, crucial for degrading juvenile hormone to facilitate metamorphosis. A gene encoding cytosolic carboxypeptidase 1 (AGTPBP1) was considerably down-regulated in *Luciola leii* in response to BaP, showing that the pollutant affects carboxylesterase activity.

3 Transcriptional Regulation of Detoxification

Although it is imperative for the presence of a large arsenal of detoxification genes in the insect genome, the constitutive expression of all these genes requires high energy and resources. Insects have evolved sophisticated regulatory networks that act only in the presence of specific chemical threats which create the required transcriptional response. This inducible system allows the organism to conserve resources during unstressed conditions and deploy a powerful defense when under chemical attack. Two major pathways are known to be central to this command-and-control system.

3.1 The CncC/Keap1 Pathway

The Cap'n'collar C (CncC) / Kelch-like ECH-associated protein 1 (Keap1) pathway is a highly conserved signaling cascade that serves as the master regulator of the cellular response to oxidative and electrophilic stress. It is the insect equivalent of the mammalian Nrf2/Keap1 pathway. In a healthy state, without any tension, the transcription factor CncC will be repressed in the cytoplasm (Figure 3a). The ubiquitination and proteasomal degradation of

CncC are perpetually mediated by the repressor protein Keap1 which ensures that its levels remain low in cells. Keap1 has many cysteine residues that are highly reactive and which serve as molecular sensors.

The protein repressor Keap1 has numerous Cysteine residues that are highly reactive residues which come with relevant roles of sensing cellular stress. Keap1 with these cysteine sensors is covalently altered by many xenobiotics, or the reactive oxygen species (ROS) they produce during metabolism, and in turn are electrophiles that are capable of reacting. The alteration causes the conformational change that impairs the Keap1 capacities to ubiquinate and degrade CncC through the proteasome [21]. Consequently, newly synthesized CncC becomes stabilized and accumulation takes place, translocation of CncC into the nucleus occurs and CncC assembles with a small Maf protein in the form of a heterodimer.

The CncC/Maf heterodimer recognizes and binds to specific DNA sequences known as Antioxidant Response Elements (AREs) or Electrophile Response Elements (EpREs), which are located in the promoter regions of a vast number of target genes. Binding of the complex initiates the transcription of these genes. The CncC/Keap1 pathway regulates broad induction of CYPs, GSTs, CarEs, and ABCs under oxidative/xenobiotic stress. This pathway has been directly implicated in conferring high levels of insecticide resistance in multiple insects, including the beetle Tribolium castaneum.

CncC/Keap1 is one of the key elements of the defense mechanism of the firefly. ROS-generating genes, such as superoxide-generating NADPH oxidase (NOXc) and protective heat shock proteins (HSP18, HSP100), a typical marker of an inactivation of CncC/Keap1, were highly up-regulated by BaP exposure in Luciola leii. Likewise, there is additional functional evidence of DEGs related to the manifestation of peroxidase that performs its functions to destroy reactive oxygen species caused by hypoxia in A. leii [22]. In Aquatica wuhana exposed to BaP, miRNA targets of oxidative stress pathways were enriched, which suggests that CncC/Keap1 is regulated. Up-regulation of 17 Aquatic Adaptation-Related Metabolites (AARM) with established antioxidant functionality (e.g., oligopeptides, cynaroside A, pokeberrygenin, inosinic acid) in Aquatica leii offers a good functional rationale of stimulation of the downstream targets of this pathway in response to the effect of hypoxia-generated ROS.

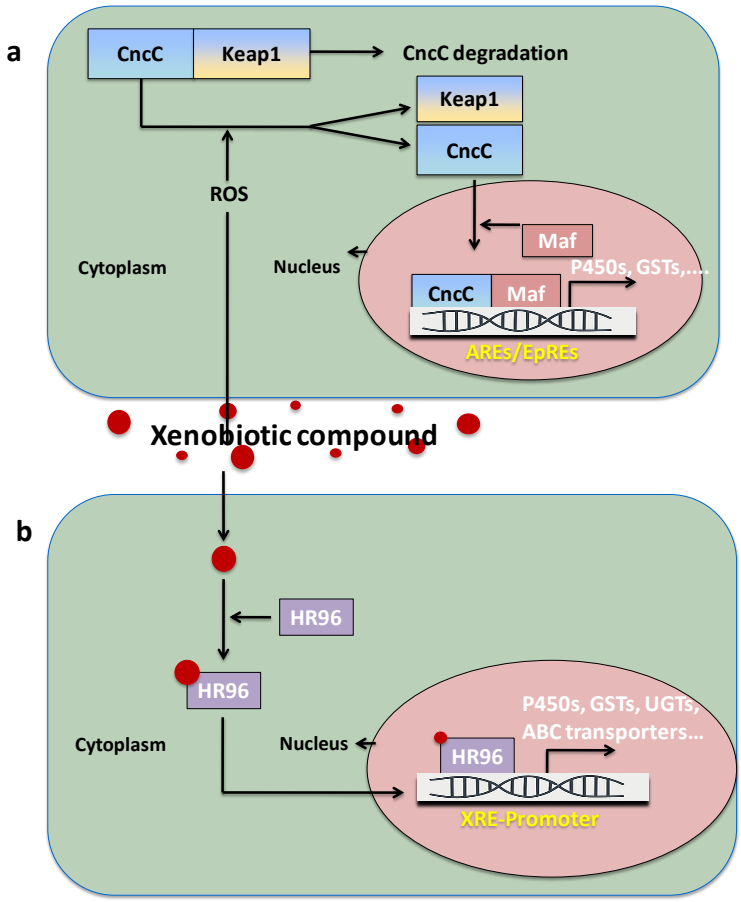

Figure 3: Schematic representation of key transcriptional regulatory pathways mediating xenobiotic response in insects

In **Figure 3,** (a) CncC/Keap1 pathway: Under basal conditions, CncC is bound to Keap1 in the cytoplasm, resulting in its ubiquitin-mediated degradation. Upon exposure to xenobiotics or reactive oxygen species (ROS), CncC dissociates from Keap1 and translocates into the nucleus, where it forms a heterodimer with Maf. The CncC–Maf complex binds to antioxidant response elements (AREs/EpREs) to activate the transcription of detoxification genes such as P450s and GSTs. (b) HR96 pathway: Xenobiotic compounds interact with the nuclear receptor HR96, facilitating its activation and translocation into the nucleus. HR96 binds to xenobiotic response elements (XRE-promoters) to induce the expression of detoxification-related genes, including P450s, GSTs, UGTs, and ABC transporters.

3.2 Nuclear Receptors (NRs)

While the CncC pathway provides a general, robust defense, the Nuclear Receptor (NR) superfamily provides a mechanism for a more specific response to chemical signals. NRs are ligand-activated transcription factors. They have a more varied ligand-binding domain (LBD) and a highly conserved DNA-binding domain (DBD). In their inactive state, they are bound by co-repressor proteins. When a chemical (a ligand) enters the cell and binds to the LBD, it causes a conformational shift that releases the co-repressors and activates the co-activator proteins. To regulate transcription, the activated NR complex binds itself to specific DNA sequences in the promoters of its target genes known as hormone response elements, or HREs (Figure 3b).

This process has been extensively investigated in vertebrates. The Constitutive Androstane Receptor (CAR) and Pregnane X Receptor (PXR) serve as "xenosensors." Because of their large and promiscuous LBDs, they can bind to a wide variety of exogenous compounds, including medicines, environmental contaminants, and natural substances. Their activation significantly increases the production of critical detoxification genes, such as CYP3A P450s.

While insects lack direct orthologs of the vertebrate PXR and CAR, the nuclear receptor DHR96 (and its orthologs across Insecta, often referred to as HR96) has been firmly established as a key functional analog. This receptor acts as a broad-scope xenosensor that is activated by diverse ligands, including phenobarbital and other chemicals, to regulate detoxification gene expression. When a chemical ligand binds to the LBD, it causes a conformational shift that releases co-repressors and recruits co-activator proteins. The activated NR complex then binds to specific DNA sequences known as hormone response elements (HREs) in the promoters of its target genes to regulate transcription.

A transcriptomic study in the firefly provides crucial clues about NR-mediated detoxification. In Aspisoma lineatum, juvenile hormone metabolism genes abundant in the lantern and fat body indirectly point to endocrine/nuclear receptor involvement in detox and light regulation [23]. Hormone receptors (HR96, EcR, USP, etc.) sense ligands and coordinate detox gene expression, integrating developmental signals with xenobiotic responses. The coordinated up-regulation of multiple CYP gene families (CYP3, CYP6, CYP9) in Luciola leii in response to BaP is a hallmark of xenobiotic-activated nuclear receptors (e.g., the AHR pathway in vertebrates).

Table 1 compares the core transcription factors and target gene families regulated by the CncC/Keap1 and NR pathways, elucidating the division of labor and synergistic effects between the two pathways.

Table 1: Detoxification genes regulated by CncC/Keap1 and NR pathway

Regulating pathways	Key transcription factors	Target gene family	Functional Description
CncC/Keap1	CncC Maf heterodimer	CYPs, GSTs, CarEs, ABCs	In response to oxidative/xenobiotic stress, extensive induction of detoxification gene expression enhances toxin tolerance.
Nuclear Receptor (NR)	HR96	CYP3, CYP6, CYP9, UGTs,ABCs	Identify specific chemical signals (such as insecticides), activate target gene expression, and achieve precise detoxification regulation.

4 Crosstalk and Integration of Regulatory Pathways

The regulation of detoxification is not managed by isolated pathways but by a complex, integrated network that allows for a nuanced response to chemical challenges. Promoters of major detoxification genes contain multiple binding sites for multiple transcription factors that allow a synergistic or combinatorial control. This design pattern is known in model insects, where the promoters of major detoxification genes contain binding sites for both CncC and a nuclear receptor such as DHR96. This dual-input module allows the insect to mount a synergistic response and strong expression in the face of a toxin because it can detect two features simultaneously: the general cellular stress that accompanies a toxin (mediated by

CncC) and the specific chemical itself (mediated by DHR96).

Although the promoter architecture of firefly detoxification genes has not been partially unfolded, transcriptomic data on it effectively support a similar co-regulatory logic. As an example, exposure of Luciola leii to benzo[a]pyrene concurrently activates the expression of both classic CncC targets (e.g., heat shock proteins and GSTs) and hallmark NR targets (e.g., a variety of CYP6 and CYP9 P450s) signifying that both pathways get co-activated to initiate a cohesive defense [24].

Although it is clear that CncC/Keap1/HR96 is at its core, a bigger perspective shows that this network has further implications, including, though not limited to, the integration with other essential systems, including immune surveillance. Massive detoxification and immune reaction in the firefly Pyrocoelia rufa to exposure to the insecticide imidacloprid, as an example, indicates the coordination of xenobiotic defense and pathogen defense pathways. It is this combined network that allows the firefly to react in a completely sarcastically calibrated response to the nature and magnitude of the chemical threat and in a successful and metabolically efficient manner to the toxin and any other possible secondary infections.

5 Future Directions and Applications

The transcriptomic and regulatory insights gained from firefly research open several avenues for advanced study and practical application. Key future directions include the experimental validation of these findings and their application in genomics and ecotoxicology.

5.1 Experimental Validation of the Regulatory Network

It is necessary to apply contemporary genetic tools to translate correlational transcriptomic information into ascertained biological activity. Insect cell lines can be used to determine important regulatory motifs and also to verify their induction by the action of particular transcription factors by means of promoter-reporter assays, in which the regulatory region of a candidate detoxification gene is fused to a reporter such as luciferase [25]. Moreover, subsequent methods including Chromatin Immunoprecipitation sequencing (ChIP-seq), which might be combined with epitope tagging in the form of CRISPR-based epitope tagging (CETCh-seq), could provide a picture of the global binding location of principal transcription factors, such as CncC or the firefly ortholog of the HR96. This would give a clear and high-resolution map of the detoxification regulatory circuit in fireflies.

5.2 Contributions to Comparative and Functional Genomics

The most abundant order of beetles (Coleoptera) on the planet is also the one the most underrepresented by our molecular knowledge on how these insects might detoxify themselves, yet the red flour beetle (Tribolium castaneum) and the Colorado potato beetle (Leptinotarsa decemlineata) are some of the greatest pests. The Lampyridae family can be a good example to address this gap in knowledge [26, 27]. Such data would be of incalculable use to comparative genomics, letting researchers trace the evolution of key families of detoxification genes through the much larger lineage of beetles. Moreover, functional validation can be obtained with the help of such techniques as RNA intervention (RNAi). Indicatively, silencing of a given candidate gene (e.g. a highly induced P450) can be achieved by injecting double-stranded RNA (dsRNA). In case the RNAi treated fireflies have notably high mortality in the presence of an insecticide, then it gives any conclusive results of the protecting power of the gene. Such a nerve combination of transcriptomics and functional genomics is the state of the art of substantiating the biological foundation of the effects of

toxicology.

5.3 Development of Molecular Biomarkers for Ecotoxicology

Transcriptomic information can also be used to determine which molecular reactions, in terms of their sensitivity and specificity, would be the strongest indicators of exposure to pesticides. The genes that are the most intensively upregulated (e.g. CYP6XY1 or GSTd1) can be cultivated into highly sensitive biomarkers [28, 29]. Through the use of some of these biomarker genes, and available, inexpensive technology, such as a quantitative PCR (qPCR), the researchers could determine the level of expression of these genes in fireflies collected under varied conditions (e.g., in an organic farm, in a conventional cornfield, or in a preserved nature reserve). A clear and early molecular warning of chemical stress and sub-lethal exposure would be manifested by elevated expression of the fireflies in the cornfield despite the presence of healthy insects [30].

6 Conclusion

Fireflies, ecological value and aesthetic marvels that comprise the organisms are not certain of their future in a world which is rapidly becoming more of a human affair. A profound mechanistic knowledge of the ability of insects to endure chemical stress is required to defend them and other non-target insects. This review has outlined the existing knowledge regarding the firefly xenobiotic response, which involves a strong enzymatic shell that is comprised of CYPs, GSTs, and other enzymatic detoxification superfamilies. We have also described the complex regulatory systems that regulate this shield, which is mainly regulated by conserved CncC/Keap1 and HR96 pathways.

In addition to listing these parts one can come up with an interesting speculation on the intense evolutionary roots of this system. Our working hypothesis is that present-day xenobiotic response in fireflies was formed under the influence of strong selective pressure that occurred long before the fireflies came in contact with artificial pollutants. These are inherent to their own peculiar biology: in their larval phase the incessant chemical conflict with their molluscan prey, and more decisively, the biological necessity of having their own powerful, internally produced defensive steroids the lucibufagins. The fitness to make, store and prevent auto-toxicity by such substances would have given a remorseless pressure of choice, refining a detoxification and carrying system of perfect particularity and energy.

It is the evolutionary history that is likely to offer the key when it comes to the modern firefly and its reaction to the anthropogenic chemicals. The prospects of this question, allowing the management of an ancient, internal, chemistry to have a relation with the mechanism of defence against a modern, external, threat, would offer a predictive structure of different species of firefly being more sensitive to particular types of pesticide.

To test these hypotheses and address current research deficiencies, future efforts must move beyond correlational genomics to functional validation. Specific research priorities should include:

(1) Mapping the regulatory architecture using promoter-reporter assays and ChIP-seq to definitively identify the genes controlled by CncC and HR96.

(2) Confirming gene function through RNA interference (RNAi) to establish the precise role of highly induced detoxification genes in conferring tolerance to specific toxins.

(3) Developing ecological tools by translating transcriptomic data into sensitive qPCR-based biomarkers for monitoring sublethal pesticide exposure in wild populations.

This research trajectory promises to establish a predictive framework for how endogenous biochemical pathways shape responses to exogenous threats. Elucidating these connections between deep evolutionary history and contemporary toxicology is a critical prerequisite for developing targeted interventions and safeguarding vulnerable populations against novel anthropogenic pressures.

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