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An overview of functional genomic tools in deciphering insecticide resistance

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In this short review, we highlight three functional genomic technologies that have recently been contributing to the understanding of the molecular mechanisms underpinning insecticide resistance: the GAL4/UAS system, a molecular tool used to express genes of interest in a spatiotemporal controlled manner; the RNAi system, which is used to knock-down gene expression; and the most recently developed gene editing tool, CRISPR/Cas9, which can be used to knock-out and knock-in sequences of interest.

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Introduction

Functional genomic technologies make use of the data produced by genomic and transcriptomic projects to try to elucidate the role played by genes of interest in *in vivo* systems. This can be done by systematically knocking-down, knocking-out or over-expressing specific targets. Not surprisingly, due to the vast array of functional genomic tools available, *Drosophila melanogaster* has been at the forefront of these studies. However, advances in germline transformation technologies in non-model insects and the development of technologies that do not require germline transformation have recently expanded the applicableness of functional genomics. Here we briefly review these technologies and how they have been applied to the study of the mechanisms of insecticide resistance in insect pests and disease vectors.

The GAL4/UAS system

Nearly 20 year ago Fischer *et al.* demonstrated that it was possible to make use of the yeast transcription factor GAL4 in the fruit fly *D. melanogaster* to activate the expression of a reporter gene inserted next to an upstream activation sequence (UAS) [1]. This work paved the way for the development of one of the most powerful functional genomics technologies, the GAL4/UAS system [2]. In their landmark work, Brand & Perrimon developed a binary system that allows spatiotemporal control of targeted gene expression in *D. melanogaster*. The system can be used to express any gene of interest (GOI), including lethal ones, as GAL4-drivers and UAS-GOI constructs are usually integrated in separate transgenic strains (Figure 1). The authors then took another major step forward by generating a library of driver strains expressing GAL4 under the control of random enhancer sequences found in the genome of *D. melanogaster*. By further screening this library with the help of a *UAS-LacZ* reporter line, they could identify the embryonic expression pattern driven by some of these enhancers. Since then, a vast number of 'trapped' enhancer GAL4 strains have been generated and are now available for the scientific community (for a comprehensive review of the GAL4/UAS system see [3,4]).

In pioneering work investigating the resistance of wild populations of *D. melanogaster* to dichloro-diphenyl-trichloroethane (DDT), Darbon *et al.* used the GAL4/UAS system to demonstrate that a single cytochrome P450 gene, *CYP6g1*, which was differentially expressed in a DDT resistant population, was responsible for conferring resistance to that insecticide [5]. By overexpressing *UAS-CYP6g1* under the control of a heat-shock inducible GAL4 driver (*Hsp-GAL4*) and showing that these flies became more resistant to DDT than control flies, the authors provided a clear correlation between *CYP6g1* expression and resistance to DDT. In a subsequent study, the overexpression of *UAS-CYP6g1* under the control of a tubulin GAL4 driver (*TubP-GAL4*) was used to demonstrate that, in addition to DDT, this P450 conferred cross-resistance to the organophosphorus (OP) compound malathion and to the neonicotinoid insecticides, acetamiprid, imidacloprid and nitenpyram [6]. Later it became clear that the insecticide resistance phenotype associated with *CYP6g1* was mainly due to the insertion of the long terminal repeat (LTR) of an *Accord* retrotransposon upstream of the gene, resulting in an increased *CYP6g1* expression in major detoxification tissues. To confirm the role played by the *Accord* LTR in DDT resistance, flies expressing *UAS-CYP6g1* under the control of an *Accord LTR-GAL4* driver (*6g1*HR-GAL4-*6c*) were shown to become more resistant to insecticides compared to control flies [7].

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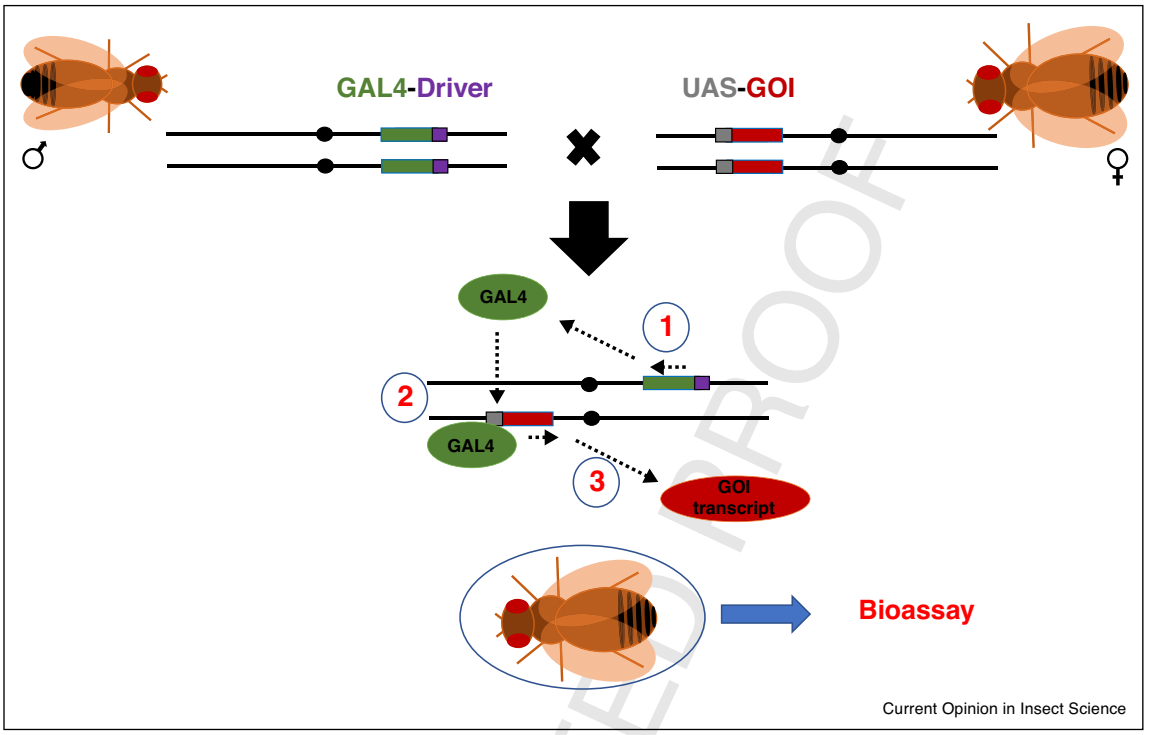
Figure 1



The GAL4/UAS binary targeted gene expression system. The system consists of a transgenic strain in which coding sequence for the yeast transcription factor, GAL4, is under the control of a promoter or enhancer of interest, Driver, and a second transgenic strain in which the GAL4 target, Upstream Activating Sequence (UAS), controls transcription of a gene of interest (GOI). GOI is only transcribed in the F1 progeny from these crosses in which one copy of each construct is present. In the F1 progeny, GAL4 is produced (1), binds to the UAS (2) and activates the expression of GOI (3). F1 flies are used in bioassays.

There are now numerous further examples of the use of the GAL4/UAS system in *D. melanogaster* to assess the contribution of individual detoxification enzymes to resistance in pest insects. GAL4-driven expression of *CYP12a4* to the midgut and Malpighian tubules of fruit flies resulted in resistance to the insect growth regulator lufenuron [8]. The GAL4 system has additionally been used to functionally validate three distinct detoxification enzymes from three biologically different pests: a carboxylesterase gene (*aE7*) conferring resistance to OPs in the Australian sheep blowfly, *Lucilia cuprina*; a glutathione S-transferase gene (*GstE2*) from the malarial mosquito, *Anopheles gambiae*, conferring resistance to DDT; and a cytochrome P450 gene (*CYP6cm1*) from the silverleaf whitefly, *Bemisia tabaci*, responsible for resistance to imidacloprid [9]. It was further employed to confirm the role of two alleles of the P450 genes *CYP6P9a* and *CYP6P9b* in driving resistance to pyrethroids in field populations of the malaria vector *Anopheles funestus* [10], and to demonstrate that overexpression of the glutathione S-transferase gene, *GSTe2*, caused resistance to DDT [11]. Moreover, the expression of the P450 gene *CYP6ER1* in transgenic flies under the control of the GAL4/UAS system demonstrated that it is responsible for strong resistance to the neonicotinoid insecticide imidacloprid in the brown planthopper *Nilaparvata lugens*, a major rice pest [12•]. A follow-up study showed that *CYP6ER1* is duplicated in resistant brown planthopper strains, with individuals carrying paralogs with and without the gain-of-function mutations responsible for conferring imidacloprid resistance [13].

Examples of the use of the GAL4/UAS system in insects other than *D. melanogaster* are rarer and the reasons for that can be related to three main constraints of non-model insects — technical difficulties of keeping large numbers of mutant stocks, unavailability of transformation technologies and husbandry protocols, and scarceness of genomic data. Despite these difficulties the technology has been developed in a few other insects. As early as 2003, Imamura *et al.* reported the establishment of a GAL4/UAS binary expression system in the silkworm *Bombyx mori* [14]. This moth-based transformation system has been further refined by studies evaluating the transcription-activation efficiency of different GAL4 variants [15] and, more recently, optimising transcriptional and translational enhancers to improve *in vivo* heterologous protein expression [16]. GAL4–UAS has also been

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