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Insect glutathione S-transferase: a review of comparative genomic studies and response to xenobiotics

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Abstract

Glutathione S-transferases (GSTs) are a superfamily of multifunctional enzymes, widely distributed in living organisms. Recently, more and more insect genome sequences are available. Genomic characterizations and comparative analyses of insect GSTs have been performed. In addition, application of high-throughput technologies, such as microarray and next-generation sequencing technology, have accelerated the identification of inducible and resistant GSTs. In this review, we mainly discussed the progress in comparative genomic analysis of insect GSTs and identification of inducible and resistant GSTs using the high-throughput technologies.

Key words: insect, glutathione S-transferase, genomics, induction, high-throughput technology.

Introduction

Glutathione S-transferases (EC2.5.1.18) are a superfamily of multifunctional isoenzymes involved in the cellular detoxification of various physiological and xenobiotic substances (Sheehan et al. 2001). Based on sequence similarity and substrate specificity, insect GST genes can be subdivided into 6 subfamilies: delta, epsilon, omega, sigma, theta and zeta. In addition, some insect genomes also exists unclassified class, which is phylogenetically related with the delta and epsilon GSTs (Lumjuan *et al*., 2007; Yu *et al*., 2008). GSTs catalyze the nucleophilic attack of the tripeptide glutathione (GSH) on electrophilic centers of toxic compounds, including insecticides, plant secondary metabolites and organic hydroperoxides (Ranson and Hemingway, 2005a; table 1). In insects, GSTs were highly related to insecticide resistance, which could directly detoxify the insecticides (table 1). In addition, insecticides entered into the body could destroy the redox balance, and cause the oxidative stress reaction and produce the the lipid hydroperoxides, such as phospholipid hydroperoxides, fatty acid hydroperoxides, 4-hydroxynonenal, etc (Giordano *et al*., 2007; Vontas *et al*., 2001; Parkes *et al*., 1993; Marnett *et al*., 2003). Some of the insect GSTs contain the non-selenium dependent glutathione peroxidases (non-SeGPx) and can eliminate the hydroperoxides. Thus, GSTs play important roles in decreasing the damages of oxidative stress produced by insecticides.

Due to the functional significance of GSTs, more and more studies were reported in insects. Meanwhile, researchers reviewed the studies on GSTs related to insecticide resistance (Ranson and Hemingway, 2005a; Enayati *et al*., 2005; Li *et al*., 2007; Ranson and Hemingway, 2005b; Che-Mendoza *et al*., 2009). Ketterman *et al*. (2011) also discussed the insecticide resistance, the polymorphic nature and structure-function studies of insect GSTs. With the complete sequencing of multiple insect genomes, it provided convenience for genomic characterization of GSTs and comparative genomic analysis. Except for the 12 species of *Drosophila* genus, GST gene annotations of other 11 species were also available (table 2). The genomic studies and comparative analyses were not well summarized. In addition, studies of induced expression profiles were a very important aspect to understand the gene functions. The high-throughput technologies were widely used to identify the inducible and resistant genes. In present review, we mainly focused on the recent progresses of genomic studies and identifications of inducible and resistant GSTs using high-throughput technologies.

Diversification of GST gene in the insect genomes

Based on the genome sequences, comparative analyses of the *Drosophila melanogaster* Meigen and *Anopheles gambiae* Giles revealed 37 and 28 cytosolic GSTs, respectively (Ranson *et al*., 2002). The GST genes of other insects were gradually characterized (table 2). Relatively, the GST gene numbers in *D. melanogaster*, *Culex quinquefasciatus* Say and *Tribolium castaneum* (Herbst) were much more than those of the other insects, and *Apis mellifera* L. contained the least gene number, only 8 members (table 2). Generally, the insect specific classes (delta and epsilon) were presented the linage-specific duplications in the most of the insect genomes, which more than half of the GSTs genes were delta and epsilon classes. The functional validations suggested that they are important when adapting to the xenobiotics (Ranson *et al*., 2002; Ranson and Hemingway, 2005a; Li *et al*., 2007). However, the complete absence of the epsilon GSTs in the *A. mellifera* and only a single delta GST may partially account for the extreme sensitivity of this species to certain insecticides (Claudianos *et al*., 2006). Although insect specific classes are important, epsilon class was also absent in several insect genomes, such as *Acyrthosiphon pisum* (Harris), *Nasonia vitripennis* (Walker) and *Pediculus*

Table 1. GST classes and its corresponding biological roles.

GST class	Biological roles (References)
Delta	Metabolism of organophosphate (Li *et al*., 2007) and organochlorine insecticides (Tang and Tu, 1994); Non-selenium dependent glutathione peroxidase activity (Sawicki *et al*., 2003)
Epsilon	Metabolism of organophosphate (Huang *et al*., 1998; Wei *et al*., 2001) and organochlorine insecticides (Ortelli *et al*., 2003); Non-SeGPx activity (Ortelli *et al*., 2003; Sawicki *et al*., 2003).
Sigma	Non-SeGPx activity (Singh *et al*., 2001; Vontas *et al*., 2001; Sawicki *et al*., 2003); Structure protein (Clayton *et al*., 1998; Ranson and Hemingway, 2005a)
Theta	Metabolism of 1-chloro-2,4-dinitrobenzene (Yamamoto *et al*., 2005)
Omega	Non-SeGPx activity (Yamamoto *et al*., 2011b)
Zeta	Participating in tyrosine degradation pathway (Board *et al*., 1997; Ranson and Hemingway, 2005a)
Unclassified	Lower non-SeGPx activity and hematin binding (Lumjuan *et al*., 2007)

Table 2. The numbers of cytosolic GSTs in the insect genomes.

Species	*D. melanogaster*	*A. gambiae*	*A. aegypti*	*C. quinquefasciatus*	*C. riparius**	*C. tentans**	*A. mellifera*	*N. vitripennis*	*T. castaneum*	*B. mori*	*T. vaporariorum**	*A. pisum*	*M. persicae**	*P. humanus*	locust*
Delta	11	12	8	17	3	2	1	5	3	4	9	10	8	4	1
Epsilon	14	8	8	10	1	0	0	0	19	8	1	0	0	0	0
Omega	5	1	1	1	1	1	1	2	4	4	0	0	0	1	0
Sigma	1	1	1	2	4	4	4	8	7	2	5	6	8	4	7
Theta	4	2	4	6	1	0	1	3	1	1	0	2	2	1	1
Zeta	2	1	1	1	1	0	1	1	1	2	1	0	0	0	0
Others	0	3	3	1	2	4	0	0	0	2	0	0	0	0	1
Total	37	28	26	38	13	11	8	19	35	23	16	18	19	11	10

Data were taken from Ranson *et al*. (2002), Strode *et al*. (2008), Friedman (2011), Claudianos *et al*. (2006), Oakeshott *et al*. (2010), *Tribolium* Genome Sequencing Consortium (2008), Yu *et al*. (2008), Ramsey *et al*. (2010), Karatolos *et al*. (2011), Li *et al*. (2009), Nair and Choi (2011), Qin *et al*. (2011). In *C. quinquefasciatus*, one GST contained only C-terminal domain was not included in this table (Friedman, 2011). *Numbers based on expressed sequence tag data.

humanus L. In addition, *Chironomus riparius* Meigen, *Chironomus tentans* F., *Trialeurodes vaporariorum* (Westwood), *Myzus persicae* Sulzer and *Locusta migratoria manilensis* (Meyen) also contained none or only one member in its EST datasets, respectively.

The omega, sigma, theta and zeta class GSTs were ubiquitously distributed in organisms. Generally, each of the ubiquitous classes contained a small quantity of members (one or two) in most of the species. However, sigma class GSTs were obviously duplicated in *N. vitripennis* (8), beetle (7), whitefly (5), *A. pisum* (6) *M. persicae* (8) and *L. migratoria manilensis* (7). Structural role has been suggested for the sigma GSTs in insects, which possess a proline/alanine-rich N-terminal extension and may aid attachment to the flight muscle (Clayton *et al*., 1998). It has also been found that some of the sigma GSTs show low-level activities with the typical GST substrates, while they might have high affinities for the lipid peroxidation product 4-hydroxynonenal (Singh *et al*., 2001). Thus, these sigma duplicates might play important roles in eliminating the by-products of oxidative stress. In addition, omega GSTs were also obviously duplicated in *D. melanogaster* (5), *T. castaneum* (4) and *Bombix mori* (L.) (4).

Based on the phylogenetic analysis, some of the GST members could not classified into known classes in *A. gambiae* (3), *Aedes aegypti* (L.) (3) and *B. mori* (2), etc. These GSTs have been temporarily named unclassified class (Ranson et al. 2002). Lumjuan et al. (2007) cloned the three unclassified GSTs of *A. aegypti* and heterologously expressed in *Escherichia coli* (Migula). While two of the recombinant proteins (*GSTI1* and *GSTX1*) were constantly retained in the insoluble fraction and could not be recovered as biologically active proteins. The activities of only the recombinant *GSTX2-2* protein were characterized. It was found that *GSTX2-2* has high activities with mode substrate 1-Chloro-2,4-dinitrobenzene (CDNB) and 3,4-Dichloronitrobenzene (DCNB), but very low activity against cumene hydroperoxide. In addition, *GSTX2-2* showed affinity for hematin, which suggested a role in protecting mosquitoes against heme toxicity during blood feeding (Lumjuan *et al*., 2007). In the silkworm, one unclassified GSTs (*BmGSTu*) was cloned, and its recombinant protein was also able to catalyze the biotranslation of glutathione with CDNB (Yamamoto *et al*., 2011a). Due to phylogenetic relation to delta and epsilon classes, the function of unclassified GSTs might play important roles in adapting the special niches.

Genomic organization of insect GSTs

The duplicates of each class often show the cluster distribution in the genome. Friedman (2011) summarized the GST gene clusters among the insect genomes. It was indicated that larger clusters were observed among the dipterans and the coleopteran (Friedman, 2011). For instance, in *A. gambiae*, all the 8 epsilon GSTs are found on chromosome 3R (figure 1A), and two closely linked clusters each consisting of six genes are sequentially arranged on chromosome 2R divisions 18B and 19D (figure 1B); in *D. melanogaster*, ten members of the epsilon class are located on chromosome 2R division 55C9, while ten members of the delta class on chromosome 3R 87B (figure 1). The sequential arranged GSTs might origin by local duplications. In the *A. gambiae* and *D. melanogaster* epsilon clusters, there are evidences of recent internal duplications within the clusters. Those genes, which have probably diverged recently, are located next to each other and phylogenetically closely related (Sawicki *et al*., 2003; Ding *et al*., 2003). However, in the silkworm, eight members of epsilon class are found, but only three members are clustered on chromosome 7 (figure 1A) (Yu *et al*., 2008). It suggested that the duplication mechanism of the silkworm epsilon GSTs might different from the other insects.

Phylogeny of insect GSTs

Insect-specific delta and epsilon should be originated after the other classes, which are phylogenetically related with the theta class (Ranson *et al*., 2002; Ding *et al*., 2003; Yu *et al*., 2008; Lumjuan *et al*., 2007). In most insects, delta and epsilon classes have been experienced linage-specific expansions. However, these duplication events are not recent as they are not highly similar at the amino acid level (Friedman, 2011). Due to the linage-

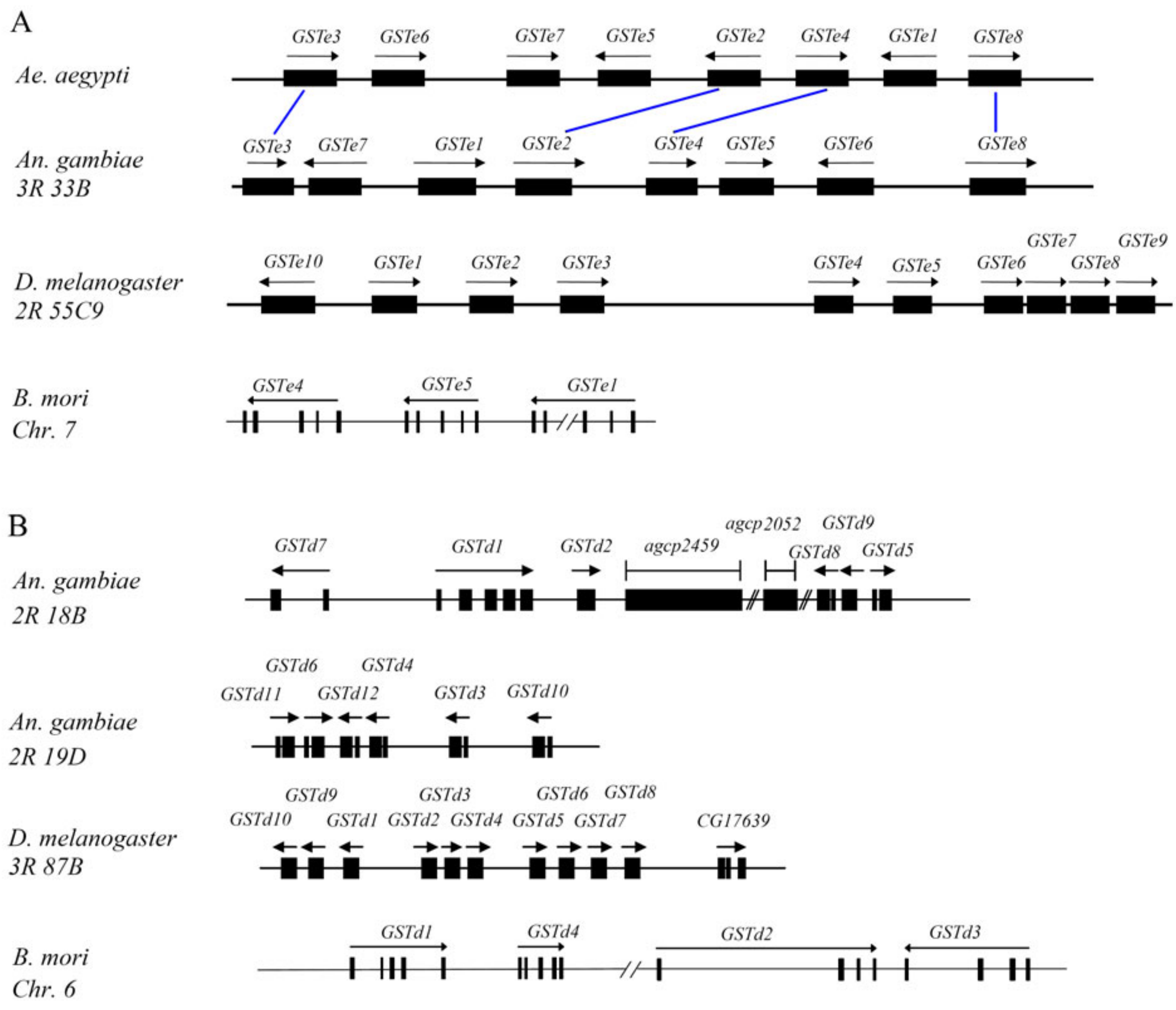

Figure 1. Tandem distributions of delta and epsilon GSTs in some insect genomes: A) epsilon class, B) delta class. *B. mori* (Yu *et al*., 2008), *A. gambiae* and *D. melanogaster* (Ding *et al*., 2003), *A. aegypti* (Lumjuan *et al*., 2007).

specific duplication, the characterization of orthologous genes among insects are very difficult (Ranson *et al*., 2002). In the delta and epsilon classes, only two secure 1:1:1 orthologs are identified among *A. aegypti*, *A. gambiae* and *D. melanogaster* (Lumjuan *et al*., 2007). Furthermore, single secure 1:1:1 orthologous relationships are identified among *A. gambiae*, *D. melanogaster* and *B. mori* (Yu *et al*., 2008). For the other four classes, based on the phylogenetic analysis, Friedman speculated that the origins of those classes predate the vertebrate and insect divergence (Friedman, 2011). Enayati *et al*. (2005) pushed the origins of the zeta and theta classes to before the origins of multicellular life.

The synteny analyses of GSTs have been conducted among some species. For instance, both *A. gambiae* and *A. aegypti* epsilon clusters consist of eight members, four putative orthologs are identifiable between the species (Lumjuan *et al*., 2007). The synteny evidence is also found between *D. melanogaster* and *A. gambiae*. Friedman (2011) found that ten members (*GSTe1* to *GSTe10*) of *D. melanogaster* and seven members (*GSTe1* to *GSTe7*) of *A. gambiae* were phylogenetically related respectively. In addition, Severson *et al*. (2004) showed evidence that the distribution regions of epsilon GSTs, *D. melanogaster* chromosome 2R division 55C9 and *A. gambiae* chromosome 3R, are syntenic at the chromosomal level. Combining these evidences, the two GST clusters of *D. melanogaster* and *A. gambiae* might share a common ancestor (in an ancient dipteran or earlier insect ancestor) even though their gene expansions occurred independently (Friedman, 2011).

The elevated expression of GST genes induced by insecticides

To validate insecticide-resistant GST genes requires both biochemical evidence that GST activity has increased or that a given GST is capable of metabolizing certain insecticide, and genetic evidence that loss or overexpression of the GST changes the resistance phenotype. However, the lack of good biochemical and genetic evidence concerning the specific role of various insect GSTs in resistance is a major barrier to our understanding of insecticide detoxification. In order to validate the resistant GST genes, we could identify the candidate GSTs at first, and then characterize at biochemical and genetic level. Identification of inducible GSTs is an important way to find the candidate GSTs conferring the insecticide resistance. Therefore, more and more studies have been focused on the characterization of inducible GSTs (Deng *et al*., 2009; Yu *et al*., 2011; Yamamoto *et al*., 2011c; Zhao *et al*., 2010; Lumjuan *et al*., 2005; 2011). For example, in *Spodoptera litura* (F.), a bioinsecticide, *Bacillus thuringiensis* Berliner (Bt), and five synthetic chemical insecticides, 1-naphthyl methylcarbamate (carbaryl), 1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane (DDT), tebufenozide (RH5992), malathion and deltamethrin, were tested for their effects on expression of *SlGSTe2* and *SlGSTe3* in the 3rd instar by reverse-transcription PCR (RT-PCR) (Deng *et al*., 2009). After xenobiotics exposure, expression of *SlGSTe2* was up-regulated by carbaryl, DDT, deltamethrin and RH5992, and *SlGSTe3* was slightly up-regulated by carbaryl and DDT.

Insecticides are not only directly toxic to cell, but also induce oxidative stress during metabolizing (Abdollahi *et al*., 2004). Some of the insect GSTs contain the activity of glutathione peroxidase and can remove highly reactive electrophilic lipid hydroperoxide, such as 4-hydroxy-2-nonenal (4-HNE) (Singh *et al*., 2001; Vontas *et al*., 2001; Sawicki *et al*., 2003; Parkes *et al*., 1993; Ding *et al*., 2005). The GPx activity has been mainly detected in delta and epsilon GSTs (Ding *et al*., 2005; Sawicki *et al*., 2003; Ortelli *et al*., 2003). Whether or not ubiquitous GSTs also contain the detoxification roles and can be induced by xenobiotics? After exposure of herbicide glyphosate and insecticide permethrin, expression of the *BmGSTs2* gene increased noticeably in the midgut and reached a peak at 6 to 12 h in the silkworm, suggesting that the induction of *BmGSTs2* is part of the defense mechanism against exogenous chemicals (Gui *et al*., 2009). In the silkworm, *BmGSTz2* can be induced after dichlorvos and deltamethrin exposure (Zhao *et al*., 2010). Yamamoto *et al*. (2011b) found that the amounts of *BmGSTo2* mRNA produced after treatment with diazinon, permethrin and imidachloprid were 3.3-, 5.9- and 6.2-fold greater, respectively. In addition, *D. melanogaster DmGSTS1-1* and *B. mori BmGSTo2* showed the catalytic functions in conjugation of lipid peroxidation end products, suggesting that they possess the activities for GSH peroxidase (Singh *et al*., 2001; Yamamoto *et al*., 2011b). These studies suggested that the ubiquitous GSTs induced by xenobiotics might play impportant roles in protecting against oxidative stress.

Inducible or resistant GST genes identified by DNA microarray

Metabolic resistance is one of the machanisms for adapting to xenobiotics, which is mainly associated with three enzyme families: cytochrome P450 monooxygenases (P450s), carboxylesterases (COEs), and GSTs (Ranson *et al*., 2002). Insect genomes often contain large numbers of detoxification genes. It becomes very important for researchers to identify and validate the inducible and resistant genes effectively. With the development of the high-throughput technologies to detect gene expression, it provides convenience for identification of insecticide-resistant genes. DNA microarray is one of the technologies and has been widely used to identify the inducible and resistant GSTs.

Recent years, various detoxification chips have been made. In 2003, a first detoxification microarray of *D. melanogaster* was constructed, which is constituted of 132 genes including 90 cytochrome P450 genes, several other genes encoding metabolic enzymes, such as COEs and GSTs and several ‘housekeeping’ genes as controls (Le Goff *et al*., 2003). In 2006, the *D. melanogaster* toxicology microarray was developed, which contained 319 genes including all P450s, GSTs, COEs and some housekeeping genes as controls (Le Goff *et al*., 2006).

Based on the chip, three GST genes induced by phenobarbital and one GST gene induced by atrazine were identified in *D. melanogaster*. The *A. gambiae* detoxification chip containing 230 genes putatively involved in insecticide metabolism (P450s, GSTs, and COEs and redox genes, partners of the P450 oxidative metabolic complex, and various controls) have also been constructed (David *et al*., 2005). It was identified that *AgGSTE2* was elevated in the pyrethroid-resistant *RSP* strain, which has previously been implicated in dichlorodiphenyltrichloroethane (DDT)-resistant. Based on the *A. gambiae* detoxification chip, *GSTS1-2* was identified in the permethrin resistant Odumasy strain, which was over-expressed in females (Muller *et al*., 2007). Vontas *et al*. (2007) used the *A. gambiae* detoxification chip to identify the putative resistant genes in *Anopheles stephensi* Liston. Using the cross-species microarray hybridization, they found that *GSTS1-1*, *GSTS1-2* and a microsomal GSTs (*GSTMIC2*) were expressed at higher levels in the pyrethroid-resistant strain (Vontas *et al*., 2007). In 2008, *A. aegypti* detoxification chip was also developed. It was found that two epsilon GSTs were overexpressed in both PMD-R and IM resistant strains (Strode *et al*., 2008). Thus, microarray is an effective technology to identify the inducible or over-expressed resistant genes.

Characterization of inducible or resistant GSTs using next-generation sequencing technology

The microarray technology was mainly used in the model organisms, which its genomes have been sequenced. With the development of sequencing technology, transcriptome sequencing is a suitable alternative to whole genome sequencing of non-model species and can be used to characterize the resistant genes at the level of transcription (Gregory *et al*., 2011; Adelman *et al*., 2011; Karatolos *et al*., 2011; Carvalho *et al*., 2010). Using the transcriptome sequencing, it provides an extensive set of expressed sequence tags (ESTs), which can be readily adopted for the design of genomic tools such as microarray (Gregory *et al*., 2011). In addition, next-generation sequencing technology such as Roche 454-FLX platform could also allow differential gene expression analysis of the whole transcriptome between different phenotypes (insecticide resistant and susceptible species) or response to insecticides in insects (Adelman *et al*., 2011; David *et al*., 2010). Thus, next-generation sequencing technology provides a high-throughput means for identifing the resistant-related GSTs or other detoxification enzymes in non-model species.

In *Cimex lectularius* L., the transcriptomes of pyrethroid-resistant and susceptible species were sequenced using 454-FLX platform (Adelman *et al*., 2011). Analyses of newly identified gene transcripts in both Harlan (susceptible) and Richmond (resistant) bed bugs revealed that *GSTs1* was significantly over-expressed in the resistant strain, which was also validated by quantitative RT-PCR (Adelman *et al*., 2011). Similar study was also performed in *Anopheles funestus* Giles. It was indicated that differential expressions between the pyrethroid resistant laboratory strain and a pyrethroid susceptible field strain were observed for the contig corresponding to *GSTe2* with a 2.5-fold change for females and 2-fold change in pupae (Gregory *et al*., 2011). Transcriptome responses to pollutants and insecticides were also performed in the dengue vector *A. aegypti*, which four GSTs and other eight xenobiotic detoxification genes were found to be differentially transcribed (David *et al*., 2010). Among GSTs, *GSTX2* was strongly and specifically induced by the insecticide propoxur while the induction of *GSTD4* appeared less specific for xenobiotics.

Conclusion and prospect

With the development of genome sequencing technologies, a mass of insect genome sequences become available, and the GSTs have also been identified. Based on the genome sequences, the whole-genome DNA microarray or small-scale detoxificaton chips have been widely used in identification of resistant and inducible candidate GSTs. It can shorten the periods of validation of resistant GSTs. However, microarray technology also presents some defects, especially, it was mainly used in identifying the up-regulated candidates. The next-generation sequencing technology can not only identify the up-regulated genes, it can even find the target site resistance genes and mutation of important residues affecting the enzyme activity. Thus, next-generation sequencing technology might get wide usage in identification of resistant and inducible genes.

Che-Mendoza *et al*. (2009) reviewed the molecular mechanism responsible for elevated GST activity in mosquito, which is mostly due to regulatory changes that increases its transcriptional rate (see also Enayati *et al*., 2005; Ranson and Hemingway, 2005a). In recent ten years, advances of genomic and high-throughput technologies have obviously promoted the functional cognition of insect GSTs. And a large number of inducible and resistant GSTs were identified. However, little is known in regards to the molecular mechanism responsible for elevated GST expression. In order to elucidate the functions of inducible GSTs, the xenobiotic-inducible promoters need to be further studied.

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