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Molecular Cloning and mRNA Expression a Glutathione S-Transferase cDNA from the Spider, *Araneus ventricosus*

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A fat body-specific glutathione S-transferase cDNA was cloned from the spider, *Araneus ventricosus*. The cDNA encoding *A. ventricosus* glutathione S-transferase (*AvGST*) is 645 base pairs long with an open reading frame of 215 amino acid residues with a calculated molecular weight of approximately 24 kDa. Northern blot analysis showed the tissue-specifically expression of *AvGST* in the *A. ventricosus* fat body.

Key words: *Araneus ventricosus*, cDNA, Fat body, Glutathione S-transferase, Spider

Introduction

Glutathione S-transferases (GSTs; EC 2.5.1.18) are a supergene family of multifunctional enzymes that catalyze the conjugation of glutathione (GSH) to a variety of electrophilic substances including carcinogenic, mutagenic, and toxic compounds (Mannervik and Danielson, 1988; Armstrong, 1991, 1997; Hayes and Pulford, 1995). The GSTs have a main function as a detoxification enzyme that protects the tissues against electrophilc toxicants by catalyzing their conjugation with GSH.

Electrophilic substrates for GST include insecticides, hervicides, chemptherapeutic agents, and other xenobiotics. GSTs also play a vital role in protecting tissues against oxidative damage and oxidative stress (Pickett and Lu, 1989; Daniel, 1993; Hayes and Pulford, 1995; Vontas *et al.*, 2001). The mammalian GST subunits are classified on the basis of sequence, activity, inhibitors, and immunological relationships into alpha, mu, pi, theta, sigma, and microsomal classes (Meyer *et al.*, 1991; Buetler and Eaton, 1992; Mannervik *et al.*, 1992; Daniel, 1993).

In insects, GSTs are primarily of interest because of their role of insecticide resistance (Motoyama and Dauterman, 1980; Fouriner *et al.*, 1992; Snyder *et al.*, 1995; Vontas *et al.*, 2001). Molecular biological and biochemical characterization of GSTs have been intensively studied in point of their role in the detoxification of many insecticides (Wang *et al.*, 1991; Fournier *et al.*, 1992; Ranson *et al.*, 1997; Huang *et al.*, 1998). As in mammals, GST activity also appears to have major roles in the protection of insects from xenobiotics. Recently, some reports demonstrated that microsomal GST in insects is capable of transcriptionally responding to oxidative stress and/or xenobiotics (Kelner *et al.*, 2000; Toba and Aigaki, 2000). In order to obtain molecular information of the spider, *Araneus ventricosus*, we previously constructed the cDNA library using *A. ventricosus* whole bodies (Chung *et al.*, 2001). A GST homologue cDNA was isolated by screening EST (expressed sequence tag) of the *A. ventricosus* cDNA library. In this study, we report the cDNA sequence of the putative GST gene from *A. ventricosus* and compared the amino acid sequences with other GSTs. Also, we found a tissue-specific expression of the *A. ventricosus* GST in the fat body.

Materials and Methods

Animals

The spider, *Araneus ventricosus*, was collected at Namhae, Kyungnam province in Korea. The live spider was directly used in this study.

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cDNA library screening, nucleotide sequencing and data analysis

A cDNA library (Chung *et al.*, 2001) was constructed using whole bodies of the spider *A. ventricosus*. Sequencing of randomly selected clones harboring cDNA inserts was performed to generate the expressed sequence tags (ESTs). For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega, Madison, WI). Sequence of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI (www.ncbi.nlm.nih.gov). GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program (www.ncbi.nlm.nih.gov /BLAST). MacVector (ver. 6.5) was used to align the amino acid sequences of glutathione S-transferase (GST) gene. With the fourteen GenBank-registered GST amino acid sequences, phylogenetic analysis was performed using PAUP* (Phylogenetic Analysis using Parsimony) version 4.0 (Swofford, 2000). The accession numbers of the sequences in the GenBank are as follows: *Araneus ventricosus* (this study), *Bombyx mori* (AAP50848), *Caenorhabditis elegans* (NP508625), *Dirofilaria immitis* (P46426), *Solenopsis invicta* (AAP79878), *Manduca sexta* (P46429), *Platynota idaeusalis* (AAC34079), *Blattella germanica* (O18598), *Gryllotalpa orientalis* (AAR84628), *Mus musculus* (AAK28508), *Rattus norvegicus* (NP803175), *Mesocricetus auratus* (A23732), *Musca domestica* (P46437), *Drosophila melanogaster* (P41043), and *Xenopus laevis* (AAM82563). Hydropathic analysis was done as described by Kyte and Doolittle (1982). Helical and non-helical regions of *A. ventricosus* GST were predicted by the Garnier-Robson method (Garnier *et al.*, 1978).

RNA isolation and Northern blot analysis

The spider *A. ventricosus* was dissected under the Stereomicroscope (Zeiss, Jena, Germany), individual samples such as fat body, midgut, silk gland, and epidermis were harvested, and washed twice with PBS (140 mM NaCl, 27 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4). Total RNA was isolated from the whole body, midgut, fat body, silk gland, and epidermis of the *A. ventricosus* by using the Total RNA Extraction Kit (Promega). Total RNA (10 μg/lane) from the *A. ventricosus* was denatured by glyoxalation (McMaster and Carmichael, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) and hybridized at 42°C with a probe in a hybridization buffer containing 5 × SSC, 5 × Denhardts solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA. The 823 bp *A. ventricosus* GST cDNA clone was labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA) for use as a probe for hybridization. After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) at 65°C and exposed to autoradiography film.

Results and Discussion

Cloning, sequencing and phylogenetic analysis of *A. ventricosus* GST cDNA

A cDNA library was constructed using whole bodies of *A. ventricosus* (Chung *et al.*, 2001). Sequencing of randomly selected clones harboring cDNA inserts was performed to generate the *A. ventricosus* ESTs. One clone, which is 825 bp long had a full-length coding sequence similar to that of previously reported GSTs. The nucleotide and deduced amino acid sequences of a cDNA encoding the A. ventricosus GST (*AvGST*) are presented in Fig. 1. The *AvGST* cDNA contains an open reading frame (ORF) of 645 bp encoding 215 amino acid residues with a calculated molecular weight of approximately 24 kDa.

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-53          GCGCACTCATATTTCGAATCGAAGGCGCTCGCAACAGAACATACTGAAAAGCC
  1  ATGCTAAATACAAGATCATCGATTATGTGTATCCCACAACAGGAGAATTGGCCAGACTC
  1  M  A  K  Y  K  I  I  D  Y  V  Y  P  T  T  G  E  L  A  R  L

 61  ATACTACAGTACAAAGATGTCGAGTACGATGATGAAAAAGTTGATTCGCCAGACAGATTA
 21  I  L  Q  Y  K  D  V  E  Y  D  D  E  K  V  D  S  P  D  R  L

121  TACGAAGCCGAAGAAGATTCTCCATTTGGCGTGTTACCAATTCTGCTAGTTGATGGAAAA
 41  Y  E  A  E  E  D  S  P  F  G  V  L  P  I  L  L  V  D  G  K

181  GTAGTCGCTCAACAAAATGGAATTCCCCGATACTTAGCCAGAGAATTGGATCTCATTGGT
 61  V  V  A  Q  Q  N  G  I  P  R  Y  L  A  R  E  L  D  L  I  G

241  AGAAATAACGAAGAGGCCGCTATTTGCGATATGGTCATGGAGATGGCTTTGGGTGTCATG
 81  R  N  N  E  E  A  A  I  C  D  M  V  M  E  M  A  L  G  V  M

301  TTCTCTAAAGCTGAGAGGAAACCAAATCTCGAAGCTCGATGCCAACAACAGATCGCTCTA
101  F  S  K  A  E  R  K  P  N  L  E  A  R  C  Q  Q  Q  I  A  L

361  TTCAAACAAATGATTGAAGAAGACGTTCCAAGATTCTTAGCAAAGTACGAGAGGTTCCTA
121  F  K  Q  M  I  E  E  D  V  P  R  F  L  A  K  Y  E  R  F  L

421  GAAAAGTCTGATCTCAAAAGTGGATACTTAGCAAGTGAACAGCTCACATGGTGTGATCTG
141  E  K  S  D  L  K  S  G  Y  L  A  S  E  Q  L  T  W  C  D  L

481  GGAGTGGCTTTGACGTTCGCTGGAATGCAAATCAGGCAACCAAAGCTCCTTGAGAAGTAT
161  G  V  A  L  T  F  A  G  M  Q  I  R  Q  P  K  L  L  E  K  Y

541  CCTCGTCTGAAACATTTCGTGGATAAGGTTACAGCTAACGATGTTGTCTCTCATTTTGTA
181  P  R  L  K  H  F  V  D  K  V  V  S  H  F  V  E  T  E  L  A

601  GAAACTGAATTAGCACCGTCAGCTGTTCTCAGGAAAAACAGCATGTAAAAAGAATAATTT
201  E  T  E  L  A  P  S  A  V  L  R  K  N  S  M  *

661  GTGTTGTTATCAATGTGATCAAAATGTTGTGTTTATTTTTTTTAGCGGATAAATGATATA
721  ATGTATAATTCTGAGAAATTTGTTCAATAAATAATTAAAACTAAAAAAAAAA
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Fig. 1. The nucleotide and deduced amino acid sequences of *A. ventricosus* GST cDNA. The start codon of ATG is boxed and the termination codon is shown by asterisk. The polyadenylation signal AATAAA is underlined. The GenBank accession numbers of *A. ventricosus* GST cDNA is AY700061.

Fig. 2. Alignment of deduced amino acid sequences of *A. ventricosus* GST with known GSTs. Residues are numbered according to the aligned GST sequences, and invariant residues are shaded black. Dots represent gaps introduced to improve alignment. The GST sequences were taken from the following sources: *A. ventricosus* (AvGST; this study), *B. mori* (BmGST2; AAP50848), *C. elegans* (CeGST-11; NP508625), *D. immitis* (DiGST; P46426), *S. invicta* (SiGST; AAP79878), *M. sexta* (MsGST2; P46429), *P. idaeusalis* (PiGST; AAC34079), *B. germanica* (BgGST; O18598), *D. melanogaster* (DmGSTs2; P41043), *M. domestica* (MdGST; P46437), *G. orientalis* (GoGST; AAR84628), *M. musculus* (MmGSTm2; AAK28508), *R. norvegicus* (RnGST-Yb2; NP803175), *M. auratus* (MaGST; A23732), and *X. laevis* (XlGST; AAM82563).

Percent similarity

Species	GenBank No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *A. ventricosus*	This study		39	44	40	38	39	38	38	33	35	38	32	31	33	33
2. *B. mori*	AAP50848	25		42	43	49	72	76	56	45	47	49	36	36	35	40
3. *C. elegans*	NP_508625	25	25		45	50	48	43	48	42	45	47	35	35	34	48
4. *D. immitis*	P46426	24	27	29		44	44	42	46	36	39	43	43	41	43	37
5. *S. invicta*	AAP79878	23	37	31	28		54	54	58	48	50	58	34	33	34	51
6. *M. sexta*	P46429	23	61	31	24	42		75	56	46	48	50	36	36	36	42
7. *P. idaeusalis*	AAC34097	23	63	26	25	38	61		54	50	52	49	34	34	33	41
8. *B. germanica*	O18598	22	45	32	29	45	42	43		54	55	67	33	33	36	51
9. *D. melanogaster*	P41043	21	32	28	20	34	33	35	40		86	48	34	33	33	42
10. *M. domestica*	P46437	21	33	29	22	35	33	36	39	83		51	36	36	36	42
11. *G. orientalis*	AAR84628	20	38	32	27	41	38	38	57	38	41		35	34	34	46
12. *M. musculus*	AAK28508	18	22	21	29	23	23	23	22	20	21	22		97	93	35
13. *R. norvegicus*	NP_803175	18	22	21	28	22	23	23	22	19	21	22	95		93	34
14. *M. auratus*	A23732	17	21	20	28	22	22	22	22	19	20	21	88	87		35
15. *X. laevis*	AAM82563	17	29	31	25	45	31	28	36	29	29	32	23	22	21	

Percent identity

Fig. 3. Pairwise identities and similarities of the deduced amino acid sequence of *A. ventricosus* GST cDNA among GST sequences.

A multiple sequence alignment of the deduced protein sequence of *AvGST* with other GST sequences is shown in Fig. 2. Alignment of the *AvGST* sequences with those of GST from several other species indicates the extent of the identity that exists. The *AvGST* showed 25% protein sequence identity to both the *B. mori* GST and *C. elegans* GST, and 24 – 20% to GST sequences from other insects (Fig. 3). A phylogenetic analysis using deduced amino acid sequences of known GST genes revealed that the deduced amino acid sequence of *AvGST* cDNA separated with *C. elegans* GST from other GSTs (Fig. 4).

Hydropathy profile and helical and non-helical regions of *AvGST* were shown in Fig. 5. The deduced *AvGST* N-terminal sequence contains a highly hydrophilic amino acid stretch that is likely to function as a cytosolic enzyme (Fig. 5A). The *AvGST* was predicted to be structure with a helix-turn-helix motif (Fig. 5B). The enzyme is believed to play an important role in cellular protection system. Several studies have suggested that the cytosolic enzymes are involved in susceptibility to carcinogenesis (Brockmoller *et al.*, 1998; Henderson *et al.*, 1998; Hengstler *et al.*, 1998; Strange *et al.*, 1998), cytotoxic drug resistance of cells (Schecter *et al.*, 1992; Tew *et al.*, 1998), insecticide resistance of insect (Wang *et al.*, 1991; Fournier *et al.*, 1992; Ranson *et al.*, 1997; Huang *et al.*, 1998), and protective role against oxidative damages to the cellular membranes (Mosialou and Morgenstern, 1989; Mosialou *et al.*, 1995; Kelner *et al.*, 2000).

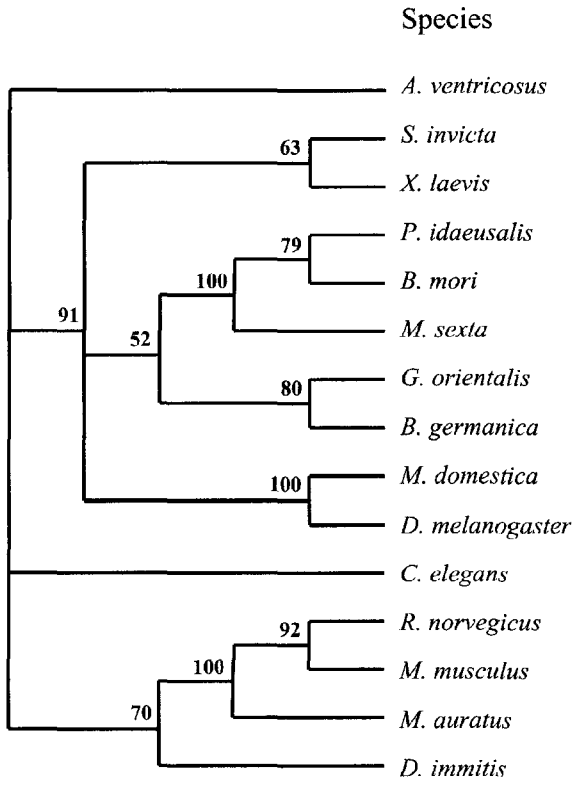

Fig. 4. Phylogenetic tree of aligned amino acid sequences of the *A. ventricosus* GST and the other known GSTs. The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1,000 replicates.

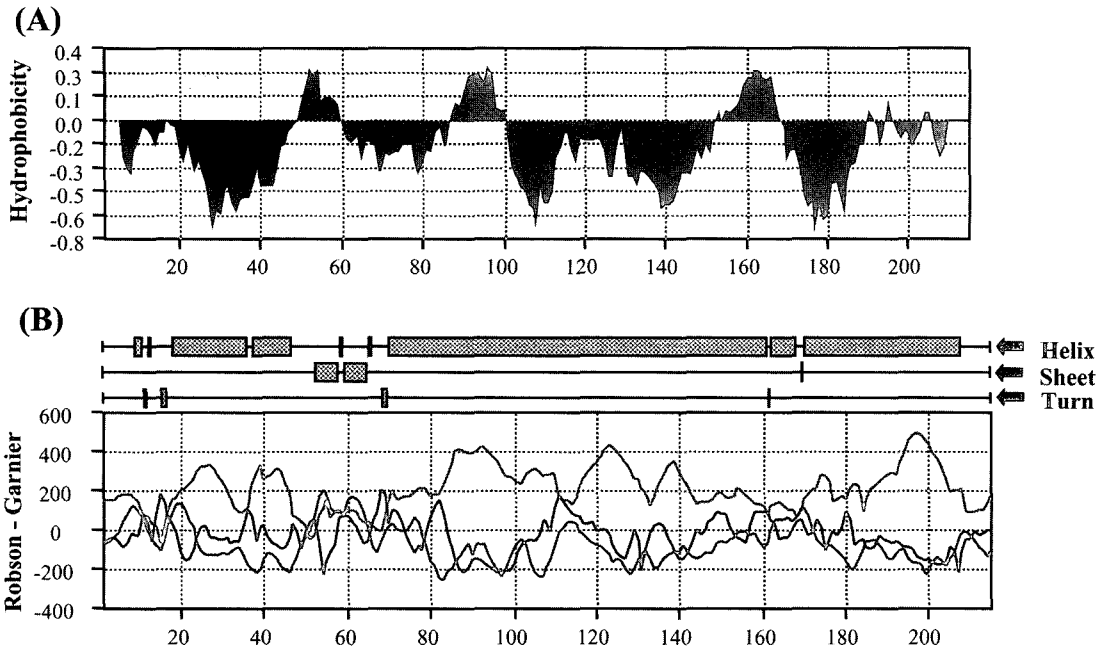

Fig. 5. Hydropathy profile (A) and predicted helical regions (B) of *A. ventricosus* GST.

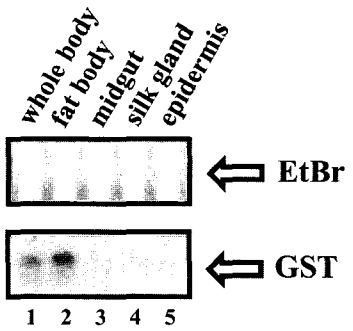

Fig. 6. Northern blot analysis of the *A. ventricosus* GST messages. Total RNA was isolated from the whole body (lane 1), fat body (lane 2), midgut (lane 3), silk gland (lane 4), and epidermis (lane 5). The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis (upper panel), transferred onto a nylon membrane, and hybridized with the appropriate radiolabelled probe (lower panel). *A. ventricosus* GST messages are indicated by arrow on the right side of the panel.

Tissue-specific expression of *A. ventricosus* GST

The tissue specific nature of *AvGST* expression was determined from fat body, midgut, silk gland and epidermis by Northern blot analysis (Fig. 6). *AvGST* was found to be expressed only in fat body and not in midgut, silk gland and epidermis of *A. ventricosus*, evidencing the fat body as a specific site for *AvGST* synthesis.

In previous reports, the insect GSTs were expressed in the midgut (Tate *et al.*, 1982; Snyder *et al.*, 1995), fat body (Chien and Dauterman, 1991), hemolymph cells and other tissues (Franciosa and Berge, 1995). In insects, GSTs play an important role in the resistance and/or detoxification to insecticides and other xenobiotics (Motoyama and Dauterman, 1980; Riskallah *et al.*, 1986; Snyder *et al.*, 1995; Yu, 1982, 1989). Microsomal GST-I (MGST-I) in *D. melanogaster* is expressed ubiquitously with the highest expression in the larval fat body (Toba and Aigaki, 2000) and is similar to mammalian membrane-bound GSTs (Toba *et al.*, 1999). It has been shown that a membrane-bound enzyme, MGST-I has a protective role against oxidative damages (Mosialou and Morgenstern, 1989; Mosialou *et al.*, 1995; Kelner *et al.*, 2000). In this study, the *A. ventricosus* GST is expressed only in the fat body. It is likely that the fat body is a site where large quantities of GST are synthesized for the protection of the cell from oxidative damages and/or xenobiotics.

In conclusion, we have cloned and characterized a novel cDNA encoding putative glutathione S-transferase from the spider *A. ventricosus*. We hope that the molecular characterization of glutathione S-transferase in *A. ventricosus* in this study will expand the understanding of invertebrate glutathione S-transferases.

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References

Armstrong, R. N. (1991) Glutathione S-transferases: reaction mechanism, structure, and function. *Chem. Res. Toxicol.* **4**, 131-140.

Armstrong, R. N. (1997) Structure, catalytic mechanism and evolution of the glutathione transferases. *Chem. Res. Toxicol.* **10**, 2-18.

Brockmoller, J., I. Cascorbi, R. Kerb, C. Sachse and I. Roots (1998) Polymorphisms in xenobiotic conjugation and disease predisposition. *Toxicol. Lett.* **102**, 173-183.

Buetler, T. M. and D. L. Eaton (1992) Glutathione S-trasnsferases: Amino acid sequence comparison, classification and phylogenetic relationship. *Env. Carcino. Ecotox. Rev.* **C10**, 181-203.

Chien, C. and W. C. Dauterman (1991) Studies on glutathione S-transferase in Helicoverpa (=*Heliothis*) zea. Insect Biochem. **21**, 857-864.

Daniel, V. (1993) Glutathione S-transferases: gene structure and regulation of expression. *Crit. Rev. Biochem. Molec. Biol.* **28**, 173-207.

Fournier, D., J. M. Bride, M. Poirie, J. M. Berge and F. W. Plapp Jr. (1992) Insect glutathione S-transferases: Biochemical characteristics of the major forms from houseflies susceptible and resistant to insecticides. *J. Biol. Chem.* **267**, 1840-1845.

Garnier, J., D. J. Osguthorpe and B. Robson (1978) Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. *J. Mol. Biol.* **120**, 97-120.

Hayes, J. D. and D. J. Pulford (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.* **30**, 445-600.

Henderson, C. J., A. G. Smith, J. Ure, K. Brown, E. J. Bacon and C. R. Wolf (1998) Increased skin tumorigenesis in mice lacking pi class glutathione S-transferases. *Proc. Natl. Acad. Sci. USA* **95**, 5275-5280.

Hengstler, J. G., M. Arand, M. E. Herrero and F. Oesch (1998) Polymorphisms of N-acetyltransferases, glutathione S-transferases, microsomal epoxide hydrolase and sulfortransferases: influence on cancer susceptibility. *Recent Results Cancer Res.* **154**, 47-85.

Huang, H. S., N. T. Hu, Y. E. Yao, C. Y. Wu, S. W. Chiang and C. N. Sun (1998) Molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the diamondback moth, Plutella xylostella. Insect biochem. *Molec. Biol.* **28**, 651-658.

Kelner, M. J., R. D. Bagnell, M. A. Montoya, L. A. Estes, L. Forsberg and R. Morgenstern (2000) Structural organization of the microsomal glutathione S-transferase gene (MGST1) on chromosome 12p13.1-13.2. *J. Biol. Chem.* **275**, 13000-13006.

Kyte, J. and R. F. Doolittle (1982) A simple method for displaying hydropathic character of a protein. *J. Mol. Biol.* **157**, 105-132.

Mannervik, B., Y. C. Awasthi, P. G. Board, J. D. Hayes, C. Di Illio, B. Ketterer, I. Listowsky, R. Morgenstern, M. Muramatsu, W. R. Pearson, C. B. Pickett, K. Sato, M. Wilderstein and C. R. Wolf (1992) Nomenclature for human glutathione transferases. *Biochem. J.* **282**, 305-308.

Mannervik, B. and U. H. Danielson (1998) Glutathione transferases structure and catalytic activity. *CRC Crit. Rev. Biochem.* **23**, 283-337.

McMaster, G. K. and G. G. Carmichael (1977) Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Natl. Acad. Sci. USA* **74**, 4835-4838.

Meyer, D. J., B. Coles, S. E. Pemble, K. S. Gilmore, G. M. Fraser and B. Ketterer (1991) Theta, a new class of glutathione transferases purified from rat and man. *Biochem. J.* **274**, 409-414.

Mosialou, E. and R. Morgenstern (1989) Activity of rat liver microsomal glutathione transferase toward products of lipid peroxidation and studies of the effect of inhibitors on glutathione-dependent protection against lipid peroxidation. *Arch. Biochem. Biophys.* **275**, 289-294.

Mosialou, E., F. Piemonte, C. Anderson, R. M. Vos, P. J. van Bladeren and R. Morgenstern (1995) Microsomal glutathione transferase: lipid-derived substrates and lipid dependence. *Arch. Biochem. Biophys.* **320**, 210-216.

Motoyama, N. and W. C. Dauterman (1980) Glutathione S-transferases: their role in the metabolism of organophosphorus insecticides. *Rev. Biochem. Toxic.* **2**, 49-69.

Pickett, C. B. and A. Y. H. Lu (1989) Glutathione S-transferases: Gene structure, regulation, and biological function. *Ann. Rev. Biochem.* **58**, 743-764.

Ranson, H., L. A. Prapanthadara and J. Hemingway (1997) Cloning and characterization of two glutathione S-transferases from a DDT-resistant strain of *Anopheles gambiae*. *Biochem. J.* **324**, 97-102.

Riskallah, M. R., W. C. Dauterman and E. Hodgson (1986) Nutritional effects on the induction of cytochrome *p*-450 and glutathione S-transferase in larvae of the tobacco budworm, *Heliothis virescens* (F.). *Insect Biochem.* **16**, 491-499.

Schecter, R. L., M. A. Alaoui-Jamali and G. Batist (1992) Glutathione S-transferase in chemotherapy resistance and in carcinogenesis. *Biochem. Cell. Biol.* **70**, 349-353.

Snyder, M. J., J. K. Walding and R. Feyerreisen (1995) Glutathione S-transferases from larval *Manduca sexta* midgut: sequence of two cDNAs and enzyme induction. *Insect Biochem. Molec. Biol.* **25**, 455-465.

Strange, R. C., J. T. Lear and A. A. Fryer (1998) Glutathione S-transferase polymorphisms: influence on susceptibility to cancer. *Chem. Biol. Interact.* **111**, 351-364.

Swofford, D. L. (2000) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4, Sinauer Sunderland, MA.

Tate, L. G., S. S. Nakat and E. Hodgson (1982) Comparison of detoxification activity in midgut and fat body during fifth

instar development of the tobacco hornworm, *Manduca sexta*. *Comp. Biochem. Physiol.* **72C**, 75-81.

Tew, K. D., M. O'Brien, N. M. Laing and H. Shen (1998) Coordinate changes in expression of protective genes in drug-resistant cells. *Chem. Biol. Interact.* **111**, 199-211.

Toba, G. and T. Aigaki (2000) Disruption of the *Microsomal glutathione S-transferase-like* gene reduces life span of *Drosophila melanogaster*. *Gene* **253**, 179-187.

Toba, G., T. Ohsako, N. Miyata, T. Ohtsuka, K. H. Seong and T. Aigaki (1999) The gene search system: a method for efficient detection and rapid molecular identification of genes in *Drosophila melanogaster*. *Genetics* **151**, 725-737.

Vontas, J. G., G. J. Small, D. C. Nikou, H. Ranson and J. Hemingway (2001) Purification, molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the rice brown planthopper, *Nilaparvata lugens*. *Biochem. J.* **362**, 329-337.

Wang, J. Y., S. McCommas and M. Syvanen (1991) Molecular cloning of a glutathione S-transferase overproduced in an insecticide-resistant strain of the housefly (*Musca domestica*). *Mol. Gen. Genet.* **227**, 260-266.

Yu, S. J. (1982) Host plant induction of glutathione S-transferase in the fall armyworm. *Pest. Biochem. Physiol.* **18**, 101-106.

Yu, S. J. (1989) Purification and characterization of glutathione S-transferases from five phytophagous lepidoptera. *Pest. Biochem. Physiol.* **35**, 97-105.