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Review

Kiyoung Kim and Jeongbin Yim*

The diverse biological functions of glutathione S-transferase omega in *Drosophila*

Abstract: Glutathione S-transferase omega (GSTO) genes in eukaryotic organisms encode proteins that are important for cell defense. However, the physiological roles of GSTOs have not been fully elucidated yet. Recently, genetic and molecular studies with *Drosophila* demonstrated that CG6781 is the structural gene of the eye color mutant *sepia* and that CG6673 is a novel genetic suppressor of the *parkin* mutant. These results provide valuable insight into the diverse functions of GSTOs in vivo. In this review, we briefly introduce recent studies and summarize the novel biological functions of GSTOs in *Drosophila*.

Keywords: ascorbic acid; dehydroascorbate; *Drosophila*; drosopterin; eye pigments; F_1F_0-ATP synthase; glutathione S-transferase omega; pyrimidodiazepine.

***Corresponding author: Jeongbin Yim,** Department of Medical Biotechnology, Soonchunhyang University, Asan, 336–745 Korea, Phone: +82-41-530-1119, Fax: +82-41-534-6702, E-mail: jyim@sch.ac.kr
Kiyoung Kim: Department of Medical Biotechnology, Soonchunhyang University, Asan, Korea

Introduction

Glutathione S-transferases (GSTs) are phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) to an electrophilic substrate. These enzymes provide protection against carcinogens, therapeutic drugs, and several types of cellular oxidative damage [1]. Based on their amino acid sequences and substrate specificities, GSTs are grouped into at least ten classes: α, δ, ε, κ, μ, π, σ, θ, ζ, and ω [2].

The GST omega (GSTO) is the most recently defined GST class [3]. The active sites of GSTOs have a cysteine residue at the N-terminus that can bind to GSH, whereas other GST classes have tyrosine or serine residues in their active sites. GSTOs have thiol transferase and dehydroascorbate (DHA) reductase activities, the latter of which is similar to the reactions catalyzed by thioredoxin and glutaredoxin [3]. In addition, GSTOs catalyze the reduction of monomethylarsonic acid [4, 5]. Human GSTO1 modulates the ryanodine receptor, which is a Ca^{2+} release channel modulator and is involved in the activation of interleukin-1β, an important mediator of the inflammatory response [6, 7]. Variations in the human *GSTO1* gene may be associated with the risk of breast cancer and hepatocellular carcinoma [8]. Furthermore, a polymorphism in the human *GSTO2* gene is associated with the risk of ovarian cancer [8, 9]. The following is a mini-review of the recent studies investigating the novel biological functions of GSTOs in *Drosophila*.

GSTO genes in *Drosophila*

Currently, the *Drosophila* GST genes are divided into six classes: δ, ε, σ, ω, ζ, and θ. *Drosophila* harbor 36 GST genes that encode 41 proteins [2, 10]. The GST δ and ε classes contain 11 and 14 genes that encode 12 and 14 proteins, respectively. The GST ζ and θ classes contain two and four genes, respectively. Four different *GSTO* genes in *Drosophila* are located on chromosome 3L 66D5: *CG6781, CG6662, CG6673*, and *CG6776*. The *CG6673* gene produces two alternatively spliced products, isoforms A and B (Figure 1). Recently, these four *GSTO* genes were named *sepia*, *GstO1, GstO2*, and *GstO3*, respectively [2]. The average sequence identities/similarities are high, at 43%–65%/66%–82%, based on the amino acid sequence alignment of the different isoforms of GSTO [11]. All isoforms of *Drosophila* GSTO have N-terminal extensions and cysteine residues in the GSH-binding site rather than tyrosine or serine residues, which are found in the active sites of other classes of GSTs. In addition, *Drosophila* GSTOs all have high thiol transferase and DHA reductase activities, characteristic of GSTOs, and low activity towards 1-chloro-2,4-dinitrobenzene (CDNB), a general GST substrate [11].

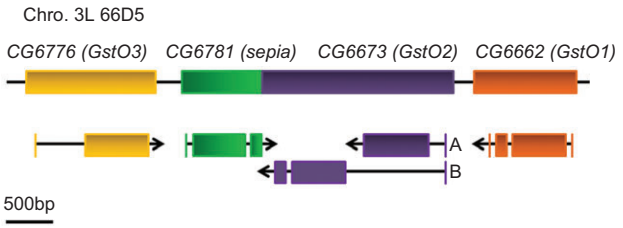

Figure 1 Genomic organization of the *Drosophila GSTO* genes.
Four *Drosophila GSTO* genes are located on chromosome 3L. The *CG6673* (*GstO2*) gene has two alternative splice variants, isoforms A and B. The arrow indicates the direction of transcription.

CG6781 is the structural gene for *sepia* and encodes pyrimidodiazepine (PDA) synthase, a key enzyme in drosopterin biosynthesis

Eye color in *Drosophila* is due to the presence of two types of pigments, the brown 'ommochromes' and the red 'drosopterins'. Drosopterins consist of five compounds, which have been referred to as drosopterin, isodrosopterin, neodrosopterin, aurodrosopterin, and fraction e [12]. The eye color mutant se^1 is defective in the PDA synthase that catalyzes the conversion of 6-pyruvoyltetrahydropterin (6-PTP) into PDA, a key intermediate in the drosopterin biosynthetic pathway [11]. Therefore, se^1 mutant flies have dark brown eyes. In a recent study, our group determined that *CG6781* is the structural gene of the *Drosophila* eye color mutant *sepia* and encodes a PDA synthase. se^1 mutant flies have dramatically decreased levels of all red eye pigments in the head. This eye pigment-defective phenotype of the se^1 mutant was rescued by the transgenic expression of CG6781 in the se^1 mutant background (Figure 2).

CG6673 regulates mitochondrial F_1F_0-ATP synthase activity in a *Drosophila* model of Parkinson's disease (PD)

It has been reported that single nucleotide polymorphisms in human *GSTO* genes are associated with the age at onset for Alzheimer's disease, PD, vascular dementia, and stroke [13, 14]. However, many studies have failed to demonstrate the molecular function of GSTOs in vivo. Recently, we found that GstO2A is a novel genetic suppressor of the *Drosophila parkin* mutant [15]. In this study, we

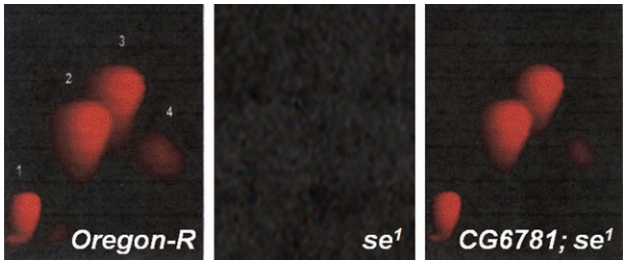

Figure 2 Transgenic GSTO expression *Drosophila* rescues eye color defect in *sepia* mutants.
Analysis of *Drosophila* eye pigments by two-dimensional thin layer chromatography (2D-TLC). The identity of each spot is as follows: 1, neodrosopterin; 2, drosopterin; 3, isodrosopterin; and 4, aurodrosopterin. Eye pigments from wild type, *Oregon-R*, heads (left panel). Eye pigments are not present in the extract of the se^1 mutant heads (middle panel). The overexpression of *CG6781, sepia*, in the se^1 mutant background rescues the phenotype of se^1 mutants (right panel).

showed compelling evidence that GstO2A catalyzes the glutathionylation of the ATP synthase β subunit, which is a catalytic component of the mitochondrial F_1F_0-ATP synthase complex (Figure 3). The glutathionylation of the ATP synthase β subunit induced by GstO2A expression in *parkin* mutants is important for the rescue of F_1F_0-ATP synthase activity in these mutants. These findings strongly suggest that enhancing the activity of GstO2A could alleviate neurodegeneration in *parkin* mutants. However, GstO2B expression was not able to rescue the defective phenotype in *parkin* mutants [15], suggesting that the two isoforms of GstO2, A and B, have different functions. Because these two isoforms differ only in the proportion of the C-terminal domain that binds the hydrophobic substrate, this region may influence substrate preference.

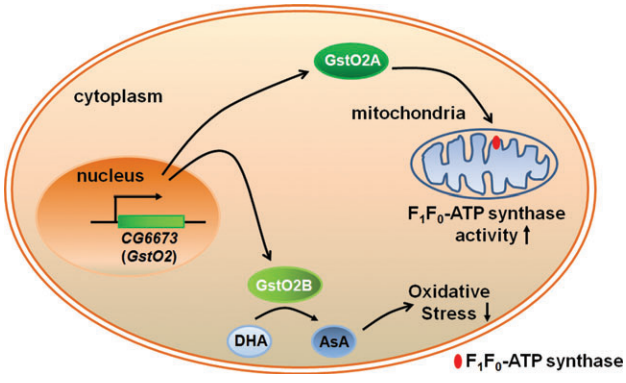

Figure 3 The proposed model for the different functions of *Drosophila* GSTOs.
CG6673 isoform A (GstO2A) regulates mitochondrial F_1F_0-ATP synthase activity through the glutathionylation of the ATP synthase β subunit. CG6673 isoform B (GstO2B) catalyzes the reduction of DHA to AsA. Reduced AsA decreases the cellular oxidative stress level.

Regulation of ascorbic acid recycling by GstO2

Ascorbic acid (AsA) is a critical cofactor in various enzymatic reactions and plays an important role in protecting cells against oxidative stress. In most cells, ascorbic acid is regenerated from the oxidized form of ascorbic acid, DHA [16, 17]. This recycling pathway of DHA to AsA is known to be mediated by GSH- or NADPH-dependent DHA reductases [18–20]. Recently, using an in vitro enzyme assay, the Board group [4] showed that the DHA reductase activity of human GSTO2 is approximately 70 to 100-fold higher than that of human GSTO1. *GstO2* has the highest GSH-dependent DHA reductase activity among the *GSTO* genes [11]. GSH-dependent DHA reductase activity is decreased in *GstO2* mutant flies. Furthermore, the AsA redox state, determined by the AsA/DHA ratio, is also dramatically decreased in *GstO2* mutants. These defective phenotypes in *GstO2* mutants can only be suppressed by the overexpression of GstO2B [15]. These data suggest that GstO2B plays a protective role against oxidative stress by regulating the AsA recycling pathway in *Drosophila* in vivo (Figure 3).

Functions of other GSTOs in *Drosophila*

The in vivo functions of *GstO1* and *GstO3* remain unknown. Interestingly, *GstO1* transcripts are highly expressed in the ovaries and testes [21] (Figure 4). The spatial expression pattern of GstO1 in adult tissues indicates that GstO1 most likely has different substrates and different functions in vivo. Studies on the molecular function of GstO1 in reproductive organs are currently underway in our laboratory.

The level of *GstO3* transcript of *Drosophila* is increased in response to heat shock, heavy metal stress, and exposure to rotenone [22–24]. These results suggest that GstO3 has a wide range of antioxidant activities. Further studies are required to understand the molecular mechanism by which GstO3 protects cells from these oxidative stresses.

Conclusion

Sequence alignment analyses revealed that GSTs, including GSTOs, exist in a wide range of organisms. This wide distribution may reflect diverse biological functions in various organisms. Previously, we provided evidence for novel diverse roles of GSTOs based on genetic and molecular studies in *Drosophila*. One of the *Drosophila* GSTOs, CG6781 (*sepia*), has been identified as the *sepia* gene. We also found that CG6673 (GstO2) is required for the activation of mitochondrial F_1F_0-ATP synthase activity and for the regeneration of AsA. The C-terminal domain of *Drosophila* GSTOs is less similar than the N-terminal domain, which contains a cysteine residue in the GSH-binding site. Therefore, the differences in the C-terminal domain, which binds to the second substrate, may be responsible for the differences in the functions of various *Drosophila* GSTOs. Further studies identifying the in vivo substrates of the *Drosophila* GSTOs will provide a better understanding of the functional

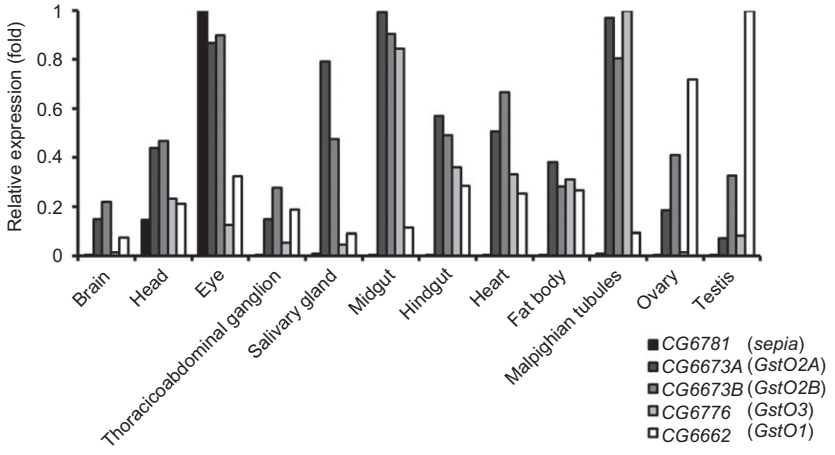

Figure 4 Tissue distribution of *Drosophila GSTO* mRNAs.
The relative mRNA expression levels of *GSTO* genes in 12 different adult tissues are shown. *CG6781* (*sepia*) mRNA is detected only in head and eye tissue. *CG6662* (*GstO1*) mRNA is primarily expressed in reproductive organs. The data on GSTO mRNA expression in adult tissues were compiled from Fly Atlas.

diversity of GSTOs. Because many biological functions are conserved in *Drosophila* and mammals, we expect that the elucidation of diverse physiological functions of *Drosophila* GSTOs will have broad biological implications.

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