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# *Caenorhabditis elegans gcs-1* confers resistance to arsenic-induced oxidative stress

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**Abstract**

Gamma-glutamylcysteine synthetase (γ-GCS) catalyzes the first, rate-limiting step in the biosynthesis of glutathione (GSH). To evaluate the protective role of cellular GSH against arsenic-induced oxidative stress in *Caenorhabditis elegans* (*C. elegans*), we examined the effect of the *C. elegans* ortholog of GCS(h), *gcs-1*, in response to inorganic arsenic exposure. We have evaluated the responses of wild-type and *gcs-1* mutant nematodes to both inorganic arsenite (As(III)) and arsenate (As(V)) ions and found that *gcs-1* mutant nematodes are more sensitive to arsenic toxicity than that of wild-type animals. The amount of metal ion required to kill half of the population of worms falls in the order of wild-type/As(V) > *gcs-1*/As(V) > wild-type/As(III) > *gcs-1*/As(III). *gcs-1* mutant nematodes also showed an earlier response to the exposure of As(III) and As(V) than that of wild-type animals. Pretreatment with GSH significantly raised the survival rate of *gcs-1* mutant worms compared to As(III)- or As(V)-treated worms alone. These results indicate that GCS-1 is essential for the synthesis of intracellular GSH in *C. elegans* and consequently that the intracellular GSH status plays a critical role in protection of *C. elegans* from arsenic-induced oxidative stress.

## Introduction

Arsenic is an environmental chemical of toxicological concern. It is a naturally occurring element, but anthropogenic activities can lead to substantial contamination of the environment. Arsenic, a known human carcinogen, is widely distributed in food, water, soil, and air. It was ranked first on The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) priority list of hazardous substances in 2003 (http://www.atsdr.cdc.gov/clist.html). Arsenic is introduced into water through the dissolution of minerals and ores. In addition, arsenic can accumulate in groundwater and well water in some areas as a result of erosion or leaching from local rocks. Moreover, industrial effluents, combustion of fossil fuels, and arsenic pesticides all contribute to arsenic contamination in freshwater systems (Tchounwou *et al*. 1999). Exposure to arsenic in drinking water represents a significant health problem for people around the world. Epidemiologic studies in Taiwan, Chile, Bangaldesh, and India have shown that arsenic exposure is associated with skin, liver, lung, bladder, and other cancers (Abernathy *et al*. 1999; NRC 1999; ATSDR 2000).

The toxicological effects of arsenic highly depend on its oxidation state, chemical composition, and bioavailability (Lerman *et al*. 1983). The trivalent form of arsenic appears to be the most toxic regardless of whether it is in an inorganic or organic form (Del Razo *et al*. 2001). The exact mechanism(s) of cellular and molecular events associated with arsenic toxicity are not well understood. While inorganic arsenic is known to be a human carcinogen, the precise mechanisms by which arsenic acts as a carcinogen in humans remain to be elucidated.

Oxidative stress results from an imbalance between free radical generation and the antioxidant defense system. Glutathione (GSH) plays a critical role in maintaining cellular redox homeostasis. GSH is also an important intracellular molecule that protects cells against endogenous and exogenous oxidative stress. Depletion of GSH by oxidants, for example, may alter the redox status of the cell and present a stressful and toxic situation. GSH levels have been reported to decrease, to increase or to remain unchanged after exposure to different metals (Eaton *et al*. 1980; Dudley & Klaassen 1984; Canesi *et al*. 1998). Arsenic exposure appears to induce oxidative stress and has been shown to affect intracellular GSH status in a number of cells (Ochi 1997). A primary target of arsenic is GSH (Cavigelli *et al*. 1996) and marked changes in GSH levels following arsenic exposure are commonly observed. Depletion of intracellular GSH in arsenite exposed cells was known to induce growth inhibition, apoptosis (Dai *et al*. 1999) and cytotoxicity (Shimizu *et al*. 1998). Moreover, studies in cultured BALB/c 3T3 cells (Ochi *et al*. 1994) and human fibroblasts (Oya-Ohta *et al*. 1996) showed that intracellular GSH plays an important role in the detoxification machinery against arsenite toxicity. These studies suggested that an increase in cellular oxidative stress could be one of the underlying mechanisms in arsenite-induced toxicity.

Glutathione is synthesized in the cell cytosol by γ-glutamylcysteine synthetase (GCS) and glutathione synthetase (Meister & Anderson 1983). GCS is proposed to catalyze the first and rate-limiting step in GSH biosynthesis (Richman & Meister 1975). Factors that regulate the expression and activity of GCS are therefore of considerable interest, as GCS appears to play a principal role in modulating glutathione homeostasis and consequently affects the capacity of the cell to withstand the deleterious effects of oxidative stress. Several studies have shown that intracellular GSH plays a principal role in protecting cells from arsenite-induced cytotoxicity *in vitro* (Huang *et al*. 1993; Ochi *et al*. 1994) and *in vivo* (Hirata *et al*. 1990). However, the role of intracellular GSH and GCS in related to arsenite-induced toxicity has not been studied in the nematode *C. elegans* yet.

cDNA clones encoding GCS have been isolated from several different eukaryotic and prokaryotic sources. In mammals, GCS is a heterodimer comprising a catalytic heavy subunit (73 kDa, GCS(h)) and a regulatory light subunit (30 kDa, GCS(l)) (Hayes & McLellan 1999). In *C. elegans*, the predicted gene, *gcs-1* is the *C. elegans* ortholog of GCS(h), a representative and well-characterized transcription factor Nrf target gene (Hayes & McMahon 2001). Recently, it has been shown that *gcs-1* expression in the intestine of *C. elegans* is induced by the transcription factor SKN-1 in response to paraquat-induced oxidative stress. (An & Blackwell 2003).

Because arsenic plays an important role in oxidative stress in biological systems, we were interested in whether arsenic-induced oxidative stress was influenced by GSH synthesis in *C. elegans*. *C. elegans* provides an excellent model system for obtaining an integrated picture of cellular, developmental, and molecular aspects of metal and metalloid toxicity (Liao & Freedman 2002). The adult hermaphrodite is composed of 959 somatic cells. The developmental and cellular biology of *C. elegans* is thoroughly understood, and the nematode contains highly differentiated muscular, nervous, digestive, and reproductive systems. Furthermore, high levels of evolutionary conservation between *C. elegans* and higher organisms are observed in many of the proteins that are induced as part of a metal-activated stress response. These include metallothione (Freedman *et al*. 1993), superoxide dismutase (Giglio *et al*. 1994), ubiquitin (Stringham *et al*. 1992), heat shock protein 70 (Heschl *et al*. 1990), glutathione *S*-transferase (Tawe *et al*. 1998), catalase (Sampayo *et al*. 2003), multidrug resistance-associated proteins (Broeks *et al*. 1996), and cadmium-responsive genes (Liao & Freedman 1998; Liao & Freedman 2001; Liao *et al*. 2002). *C. elegans* also contains homologues to many of the regulatory proteins that have been implicated in modulating the molecular response to metal exposure (Land *et al*. 1994; Kawasaki *et al*. 1999).

To evaluate the protective role of cellular GSH against the toxicity of arsenic in *C. elegans*, the effect of *gcs-1* responding to inorganic arsenic exposure was investigated. We have examined responses of *C. elegans* to both inorganic As(III) and As(V) ions as they have been implicated in the generation of reactive oxygen species (ROS) and subsequent damage to proteins and DNA. We hypothesize that inorganic arsenic exposure can disturb intracellular GSH status and hence result

in toxicity in *C. elegans*. We also hypothesize that *C. elegans gcs-1* plays a critical role in modulating GSH homeostasis and consequently the ability of the cell to withstand the deleterious effects of arsenic-induced oxidative stress. The toxic response of *C. elegans* to the different oxidation states of arsenic was also compared.

## Materials and methods

### *Chemicals*

Unless otherwise stated, all chemicals were purchased from Sigma Chemical (St. Louis, MO, USA).

### *Nematode propagation and strains*

*Caenorhabditis elegans* were grown in Petri dishes on nematode growth medium (NGM) and fed with OP50 strain *Escherichia coli* (Brenner 1974). Synchronization of worm cultures was performed as described (Hope 1999). The following strains were used: wild-type *C. elegans* N2 (var. Bristol); *gcs-1* mutant: VC337 [*gcs-1(ok436)/mIn1[mIs14 dpy-10(e128)*] which is a 837 bp chromosomal deletion (*ok436*) of the *gcs-1* locus. All nematode strains used in this work were provided by the *Caenorhabditis* Genetics Center (University of Minnesota), which is funded by the NIH National Center for Research Resources (NCRR).

### *Intracellular GSH measurement*

To measure intracellular GSH content in the wild-type and the *gcs-1* mutant, GSH assay was performed. One hundred and twenty young adult hermaphrodites of wild-type (3–4 days old) or *gcs-1* mutant (4–5 days old) nematodes were transferred from NGM plates into a 1.5 ml microcentrifuge tube containing 300 $\mu$l of lysis buffer (100 mM sodium phosphate buffer, 1 mM EDTA, pH 7.5). Worms were sonicated, then centrifuged at 10,000$\times$ *g* for 10 min. The supernatant was collected, and 5-sulfosalycyclic acid (SSA) was added to a final concentration of 1% SSA. The mixture was then centrifuged at 10,000$\times$ *g* for 10 min, and the supernatant was then collected for use in the assay. For GSH measurements, 50 $\mu$l aliquots of each sample were placed in a 96-well plate with 150 $\mu$l reaction buffer (100 mM $NaH_2PO_4$, 1 mM EDTA (pH 7.5), 0.15 mM DTNB, 0.2 mM NADPH, 1 U/ml glutathione reductase). The rate of absorbance change of the samples was measured at 405 nm at 25 °C over 2 min. Concentrations of total GSH of the sample were determined using standard curves of GSH.

### *Arsenic toxicity analyses*

Twenty young adult hermaphrodites of wild-type or *gcs-1* mutant nematodes were transferred from NGM plates into a Costar 24-well tissue culture plates containing 1 ml of K medium (53 mM NaCl, 32 mM KCl) (Williams & Dusenbery 1990) with various concentrations of As(III) or As(V) per well. Wild-type animals were exposed to 0, 0.5, 1.5, 3.0, and 4.5 mM nominal concentration of As(III) or 0, 10, 20, 40, 50, and 60 mM of As(V). The exposure nominal concentration for *gcs-1* mutant nematodes were 0, 0.025, 0.05, 0.075, and 0.1 mM of As(III) or 0, 1.5, 3.0, 6.0, and 9.0 mM of As(V). Worms were incubated at 20 °C and the dead worms were scored at different time points ranging from 2- to 24-h ($\pm$10 min). The number of dead worms was determined by the absence of touch-provoked movement when probed with a platinum wire. The tests were performed between three and six times for each concentration.

### *GSH rescue assay*

Young adult hermaphrodites of *gcs-1* mutant nematodes were pretreated with or without 50 $\mu$M GSH in K medium for 3 h. Worms were washed twice with K medium and then transferred back to NGM plates. Subsequently 20 animals from each treatment were transferred from NGM plates into Costar 24-well tissue culture plates containing 1 ml of K medium per well with various concentrations of As(III) or As(V). Worms were incubated at 20 °C and the dead worms were scored after 24 h ($\pm$10 min). The number of dead worms was determined as described above. The tests were performed between three and six times.

### *Data analysis*

The lethal concentration ($LC_{50}$) from the toxicity assays was determined using a Probit transformation (USEPA). The experiments were

performed between three and six times for error analyses. The data were used to calculate the standard deviations. Significant differences between the data were determined using student's *t*-test. With the exception of the Probit transformations, all analyses were performed using Statistaca software (StatSoft, Tulsa, OK, USA).

## Results

### *Intracellular GSH measurement*

To determine whether *gcs-1* plays a critical role in modulating GSH homeostasis in *C. elegans*, intracellular GSH was measured in wild-type and *gcs-1* mutant nematodes. As shown in Figure 1, intracellular GSH content in *gcs-1* mutant ($0.767 \pm 0.152$ nmol/120 worms) was significantly ($P < 0.001$) lower than the value of the wild-type ($1.139 \pm 0.189$ nmol/120 worms). This is in agreement with our hypothesis that *gcs-1* regulates the synthesis of GSH in *C. elegans*.

### *Lethality tests of As(III) and As(V) and establishment of LC value*

To investigate the ability of *C. elegans* to withstand the arsenic-induced toxicity, wild-type and *gcs-1* mutant nematodes were exposed to a range of As(III) and As (V) ion concentrations and the dead worms were scored over a 24 h period. The proportion of worms surviving As(III) concentrations from 0.025 to 4.5 mM after 24 h exposure varied considerably between strains (Figure 2a). The As(III) $LC_{50}$ value for *gcs-1* mutant ($0.085 \pm 0.019$ mM) was significantly ($P < 0.01$) lower than the value of the wild-type ($1.282 \pm 0.306$ mM) (Figure 2b).

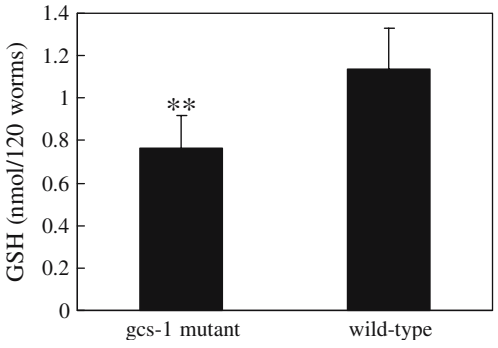


*Figure 1.* Intracellular GSH measurement. Intracellular GSH content was measured in 120 young adult hermaphrodites of wild-type or *gcs-1* mutant nematodes as described in Materials and methods. Here, we employed GSH concentrations to represent intracellular glutathione concentrations. The data are represented as means ± SD, $n = 4$. **indicates $P < 0.001$.

Similarly, *gcs-1* mutant was less resistant to As(V) ions than the wild-type strain. The proportion of worms surviving As(V) concentrations from 1.5 to 60.0 mM after 24 h exposure varied considerably between strains (Figure 3a). The As(V) $LC_{50}$ value for the *gcs-1* mutant ($2.780 \pm 0.563$ mM) was significantly ($P < 0.001$) lower than the $LC_{50}$ value of the wild-type ($34.54 \pm 3.261$ mM) (Figure 3b). As(III) exerts a higher toxic effect than that of As(V) to both wild-type and *gcs-1* mutant worms. The amount of metal ion required to kill half of the population of worms falls in order of wild-type/As(V) > *gcs-1*/As(V) > wild-type/As(III) > *gcs-1*/As(III).

### *Time-dependence of As(III) and As(V) toxicity in C. elegans*

The toxic effect of arsenic exposure to wild-type and *gcs-1* mutant worms was investigated for time dependence. Both As(III) and As(V) induced toxicity on wild-type and *gcs-1* mutant worms behaved in a time-dependent manner. The mortality rate of the nematodes to inorganic arsenic species was determined by exposing the worms to As(III) and As(V) for various time intervals as described in Materials and methods. As shown in Figure 4, the mortality rate of the wild-type worms increased as the incubation times with As(III) increased. When wild-type worms were exposed to 1.28 mM As(III) ($LC_{50}$ value), the kinetic profile showed that the survival of worms was unaffected by As(III) toxicity during the first 12 h exposure. After 12 h, the mortality rate of wild-type worms continuously increased until survival of worms was approximately 50% (Figure 4). Similarly, the mortality rate of the wild-type worms increased as the incubation times with As(V) increased. As shown in Figure 4, when wild-type worms were exposed to an As(V) $LC_{50}$ value (34.54 mM), the survival of worms was unaffected by As(V) exposure during the first 12 h. Afterwards, the mortality rate of wild-type worms continuously increased until about 50% of the worms were dead (Figure 4).

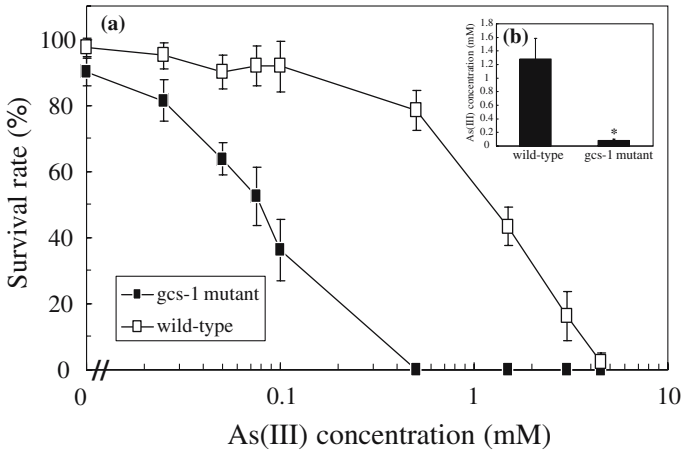


*Figure 2. C. elegans* As(III) toxicity assay. (a) Proportion of worms surviving a range of As(III) concentrations. Values are presented as a percentage of worms still alive at a particular metal ion concentration. (b) 24 h $LC_{50}$ values for As(III) based on the data shown in panel (a). Bars and standard errors represent concentrations of As(III) ions at which 50% of worms are recorded as dead. The data are represented as means ± SD, $n = 6$. *indicates $P < 0.01$.

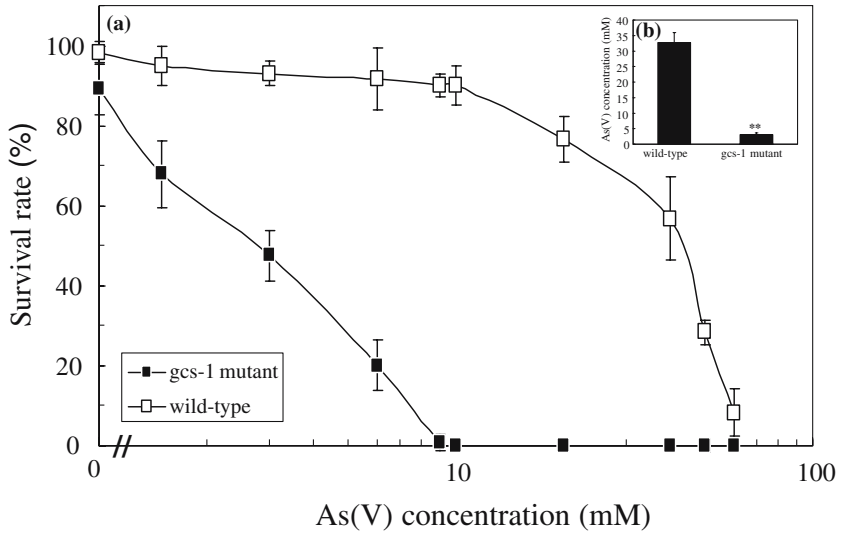


*Figure 3. C. elegans* As(V) toxicity assay. (a) Proportion of worms surviving a range of As(V) concentrations. Values are presented as a percentage of worms still alive at a particular metal ion concentration. (b) 24 h $LC_{50}$ values for As(V) based on the data shown in panel (a). Bars and standard errors represent concentrations of As(V) ions at which 50% of worms are recorded as dead. The data are represented as means ± SD, $n = 6$. **indicates $P < 0.001$.

The kinetic profiles of *gcs-1* mutant exposed to As(III) and As(V) were also shown in Figure 4. The mortality rate of the *gcs-1* mutant worms toward the exposure of these metal ions showed a time-dependence. As shown in Figure 4, the mortality rate of the *gcs-1* mutant worms increased as the incubation times with As(III) increased. *gcs-1* mutant worms appeared to be more susceptible to As(III) toxicity than the wild-type worms. When *gcs-1* mutant worms were exposed to an As(III) $LC_{50}$ value (0.085 mM), the survival rate of worms began to decrease after 4 h exposure, and the mortality rate of nematodes continuously increased until approximately 50% of the worms were dead (Figure 4). Time-dependence of As(V)-induced lethality in *gcs-1* mutant worms was also observed. As shown in Figure 4, As(V) exposure did not result in significant lethality of the animals until 4 h, after which the mortality rate of worms continuously increased until to approximately 50% survival of the worms were alive.

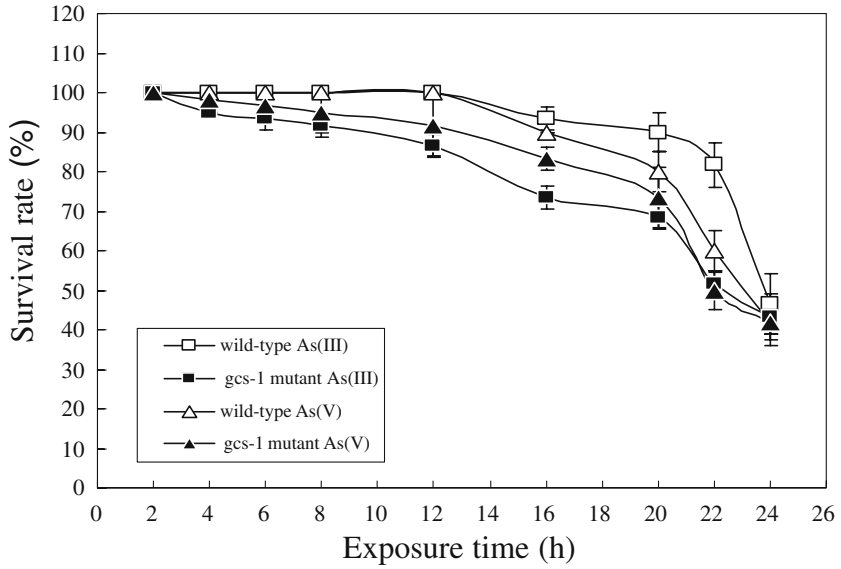


*Figure 4*. Time-dependent lethality of nematodes exposed to As(III) and As(V). Wild-type and *gcs-1* mutant nematodes were exposed to As(III) and As(V) at its corresponding $LC_{50}$. For As(III), the $LC_{50}$ values for the wild-type and *gcs-1* mutant worms are 1.282 and 0.085 mM, respectively. For As(V), the $LC_{50}$ values for the wild-type and *gcs-1* mutant worms are 34.540 and 2.780 mM, respectively. □, wild-type worms exposed to As(III); ■, *gcs-1* mutant worms exposed to As(III); Δ, wild-type worms exposed to As(V); ▲, *gcs-1* mutant worms exposed to As(V). The data are represented as means ± SD, $n = 6$.

*GSH rescues gcs-1 mutant*

Glutathione is the key metabolic intermediate downstream of γ-GCS. GSH was tested over a range of 50–1000 μM to determine the maximum levels that worms could tolerate with normal growth. The optimal GSH concentration was 50 μM; concentrations above this level had detrimental effects on worm growth (data not shown). To determine whether the addition of GSH could rescue the *gcs-1* mutant, young adult *gcs-1* mutant worms were pretreated with or without 50 μM GSH for 3 h and then worms were washed and exposed to various concentrations of As(III) or As(V). As shown in Figure 5, GSH rescued the lethal effect caused by the γ-GCS gene knockout. The addition of GSH significantly raised the survival rate of *gcs-1* mutant worms compared to As(III)- or As(V)-treated worms alone (Figure 5).

## Discussion

In this paper, we demonstrate that GCS-1 is essential for the synthesis of intracellular GSH in *C. elegans* and consequently that the intracellular GSH status plays a critical role in protection of *C. elegans* from arsenic-induced toxicity. It is well-recognized that intracellular GSH is an important endogenous antioxidant against the action of toxic xenobiotics (Pompella *et al.* 2003). Several studies have shown that intracellular GSH plays a principle role in protecting cells from arsenite-induced cytotoxicity *in vitro* (Huang *et al.* 1993; Ochi *et al.* 1994) and *in vivo* (Hirata *et al.* 1990). However, the role of intracellular GSH and GCS in relation to arsenite-induced toxicity has not been studied in the nematode *C. elegans* yet. Our study shows that GSH level in *gcs-1* mutant worms was markedly lower than that of wild-type animals (Figure 1). We wished to correlate the rescue of *gcs-1* mutant viability on medium supplemented with GSH. Pretreatment with GSH significantly raised the survival rate of *gcs-1* mutant worms compared to As(III)- or As(V)-treated worms alone (Figure 5). Although the survival rates of the *gcs-1* mutant worms were not restored to wild-type levels, it is notable that the survival of *C. elegans* can be restored when *gcs-1* mutant worms were pretreated with 50 μM GSH for 3 h (Figure 5). Therefore, the addition of GSH to medium rescued the *gcs-1* mutant worm death phenotype providing further evidence that GSH is an essential metabolite in these animals.

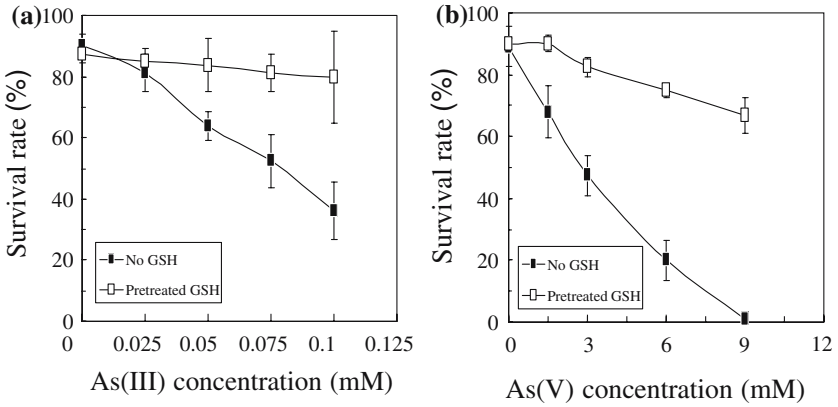


*Figure 5*. GSH rescue assay. *gcs-1* mutant nematodes were pretreated with or without 50 $\mu$M GSH for 3 h and then worms were washed and exposed to various concentrations of As(III) or As(V). Worms were incubated at 20 °C and the dead worms were scored over a 24 h period. The number of dead worms was determined as described in Materials and methods. (a) Proportion of *gcs-1* mutant worms surviving a range of As(III) concentrations with or without GSH pretreatment. (b) Proportion of *gcs-1* mutant worms surviving a range of As(V) concentrations with or without GSH pretreatment. The data are represented as means ± SD, $n = 6$.

Moreover, our data show that the extent of the rescue by GSH pretreatment is the same range for both As(III) and As(V) even the toxicity of both substances differs dramatically. This suggests that As(V) was reduced to As(III) in the presence of GSH in *C. elegans*. GSH has been shown to serve as an electron donor for the reduction of AS(V) to As(III) in aqueous solutions and in erythrocytes (Scott *et al.* 1993; Delnomdedieu *et al.* 1994). In addition, the reduction of As to trivalency by GSH has been shown to be linked to the formation of arsenotriglutathione (As(III) $(GS)_3$) (Delnomdedieu *et al.* 1993). The reduction of As(V) to As(III) may also be catalyzed by As(V) reductases. It has been shown that As(V) reductases in mammalian cells catalyze the reduction of As(V) to As(III) (Radabaugh & Aposhian 2000). These enzymes are functionally homologous to the ArsC protein of the bacterial *ars* operon that reduces As(V) to As(III) (Oden *et al.* 1994). Interestingly, As(V) reductases homolog was not being identified in the *C. elegans* genome. Therefore, GSH-mediated reduction of As(V) may predominate in *C. elegans* although the detailed mechanisms remain to be elucidated.

To investigate the ability of *C. elegans* to withstand the arsenic-induced toxicity, wild-type and *gcs-1* mutant nematodes were exposed to a range of As(III) and As(V) ion concentrations and the dead worms were scored over a 24 h period. The survival rates of worms to arsenic were dose dependent (Figures 2a and 3a). These dose-dependent decreases of cellular survival rates were directly correlated with the wild-type and *gcs-1* mutant strains of worms (Figures 2a and 3a). For wild-type worms, the survival rate did not show a significant decrease until exposure to 0.5 and 20 mM As(III) and As(V), respectively. In contrast, the survival rate of *gcs-1* mutant was significantly decreased after exposure to 0.025 and 1.5 mM As(III) and As(V), respectively. This suggests that at low arsenic exposures glutathione biosynthesis via regulation of *gcs-1* provides a protective mechanism against arsenic toxicity. While other defense systems or enzymes, such as catalase and superoxide dismutase (SOD), may still be available to protect the cells from arsenic toxicity, our data still support the concept that intracellular GSH status is critical for the survival rate of *C. elegans* when subjected to arsenic exposure. In contrast, when worms are exposed to higher concentrations of arsenic, the oxidative stress induced by high concentrations might overwhelm or inhibit the antioxidative systems, including glutathione biosynthesis. Furthermore, glutathione related enzyme activities, including glutathione reductase (GR) and glutathione *S*-transferase (GST) (Schuliga *et al.* 2002), have been shown to be affected by As(III). Therefore, it is possible that in *C. elegans*, arsenic exposure affects intracellular GSH status via regulations of intracellular glutathione biosynthesis and its related enzymes.

The $LC_{50}$ values of worms for As(III) and As(V) were established. For As(III), the As(III) $LC_{50}$ value for the wild-type ($1.282 \pm 0.306$ mM) compares well with the other report (Williams & Dusenbery 1990). Additionally, the As(III) $LC_{50}$ value for the wild-type ($1.282 \pm 0.306$ mM) was significantly ($P < 0.01$) higher than the value for the *gcs-1* mutant ($0.085 \pm 0.019$ mM) (Figure 2b). When As(III) concentration was increased to 0.025 mM, the survival rate of *gcs-1* mutant was significantly decreased. This observation indicates that following the reduction of activity of the intracellular GSH system, the animals became increasingly vulnerable to As(III) exposure, even at As(III) concentrations as low as 0.025 mM. Similarly, for As(V), the As(V) $LC_{50}$ value for *gcs-1* mutant ($2.780 \pm 0.563$ mM) was significantly ($P < 0.001$) different from the wild-type ($34.54 \pm 3.261$ mM) (Figure 3b). Thus, our findings suggest that intracellular GSH plays a critical role in protecting *C. elegans* from arsenic toxicity.

The toxic effect of arsenic exposure time to *C. elegans* was investigated. Both As(III) and As(V) induced toxicity on wild-type and *gcs-1* mutant worms showed time-dependence. For both As(III) and As(V), *gcs-1* mutant worms appeared to be more sensitive to arsenic toxicity than that of wild-type (Figure 4). When *gcs-1* mutant worms were exposed to an arsenic $LC_{50}$ value, the survival rate of worms began to significantly decrease after 4 h exposure. In contrast, when wild-type worms were exposed to the corresponding $LC_{50}$ value, the animals did not show a significant decrease of survival rate until 12 h arsenic exposure (Figure 4). This observation indicates that the reduction of intracellular GSH in the *gcs-1* mutant made the worms increasingly vulnerable to arsenic exposure. Additionally, the reduction of GSH in the *gcs-1* mutant might also affect the biosynthesis or activities of glutathione-related enzymes and consequently that resulted in a dramatic increase in sensitivity against arsenic toxicity in the mutant worms.

In conclusion, we have shown that GCS-1 is essential for the synthesis of intracellular GSH in *C. elegans* and that consequently intracellular GSH status plays a critical role in protection of *C. elegans* from arsenic-induced toxicity. The exact mechanism(s) of cellular and molecular events associated with arsenic toxicity are poorly understood. In this study, we show that arsenic exerts its toxicity through the generation of reactive oxygen species (ROS) and oxidative stress. However, many possible modes of arsenic action may also contribute in part the observed toxicity. These include chromosomal abnormalities (Nakamuro & Sayato 1981), altered DNA repair and DNA methylation patterns (Kitchin 2001), altered cell proliferation (Barrett *et al.* 1989), abnormal gene amplification (Goering *et al.* 1999), and inhibition of p53 (Hamadeh *et al.* 1999) and telomerase (Chou *et al.* 2001). It has been shown that exposure of lung epithelial cells to 5 $\mu$M As(III) results in an increase in $\gamma$-GCS (Li *et al.* 2002) expression. In *C. elegans*, in order to maintain redox homeostasis in the cells and to cope with the excess of ROS produced during the arsenic-mediate oxidative stress, *gcs-1* gene expression may be activated. Although the present work show interesting insights into mechanisms of arsenic toxicity using *C. elegans* model, the molecular mechanisms by which the arsenic-induced gene regulation and expression will require further investigation.

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