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Expression, subcellular localization, and antioxidant role of mammalian methionine sulfoxide reductases in *Saccharomyces cerevisiae*

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Despite the growing body of evidence suggesting a role for MsrA in antioxidant defense, little is currently known regarding the function of MsrB in cellular protection against oxidative stress. In this study, we overexpressed the mammalian MsrB and MsrA genes in *Saccharomyces cerevisiae* and assessed their subcellular localization and antioxidant functions. We found that the mitochondrial MsrB3 protein (MsrB3B) was localized to the cytosol, but not to the mitochondria, of the yeast cells. The mitochondrial MsrB2 protein was detected in the mitochondria and, to a lesser extent, the cytosol of the yeast cells. In this study, we report the first evidence that MsrB3 overexpression in yeast cells protected them against H_2O_2-mediated cell death. Additionally, MsrB2 overexpression also provided yeast cells with resistance to oxidative stress, as did MsrA overexpression. Our results show that mammalian MsrB and MsrA proteins perform crucial functions in protection against oxidative stress in lower eukaryotic yeast cells. [BMB reports 2009; 42(2): 113-118]

INTRODUCTION

Methionine, one of two sulfur-containing amino acids, is highly susceptible to oxidation by a variety of reactive oxygen species, including hydrogen peroxide. This methionine oxidation in proteins may induce significant changes in structure and function, but can be reversed by the repair enzymes known as methionine sulfoxide reductases (Msrs) (1). Two distinct Msr families, MsrA and MsrB, have evolved to reduce free and protein-bound methionine sulfoxide to methionine in a stereospecific manner. MsrA specifically reduces the S-form of methionine sulfoxide, whereas MsrB reduces only the *R*-form. These enzymes have been generally identified as antioxidant and protein repair enzymes, and have been implicated in crucial biological and pathological processes, including aging and neurodegenerative disorders such as Alzheimer's and Parkinson's disease (2, 3).

Mammalian genomes encode a single MsrA gene, but alternatively spliced forms have also been identified (4-6). The most abundant form of MsrA harbors a typical mitochondrial signal peptide at its N-terminus, but interestingly this MsrA protein is detected in both the cytosol and mitochondria (7, 8). With regard to the mammalian MsrB proteins, three genes have been identified (9, 10). MsrB1 is a selenoprotein and is detected in both the cytosol and nucleus. The other two MsrB proteins are cysteine-containing homologues, in which cysteine replaces the selenocysteine at the catalytic site. MsrB2 is a mitochondrial protein. The human MsrB3 gene generates two forms, MsrB3A and MsrB3B, via the alternative splicing of the first exon. MsrB3A resides in the endoplasmic reticulum (ER) and MsrB3B is targeted to the mitochondria. However, in mice, only the ER-resident form has been identified, the N-terminal ER-signal peptide of which is followed by a mitochondrial signal peptide (11).

A great many studies have described the antioxidant defense function of MsrA. MsrA has been demonstrated to perform a crucial role in cell viability by providing resistance against oxidative stress in organisms ranging from bacteria to humans. In mammals, MsrA overexpression in human T-lymphocytes (12), the lens (13), and WI-38 SV40 fibroblast cells (14) protects them against H_2O_2-induced oxidative stress. Moreover, MsrA overexpression protects neuronal PC12 (15) and cardiac myocytes (16) against hypoxia/reoxygenation-induced cell death. In contrast, little has been determined regarding the function of MsrB in cellular protection against oxidative stress, although a few studies have been conducted. The siRNA-mediated silencing of each of the MsrB genes in human lens cells has been reported to increase oxidative stress-induced cell death (17). Recently, Cabreiro *et al*. reported that MsrB2 overexpression protected Leukemia cells against oxidative stress-induced cell death (18).

In this study, we assessed the antioxidant role of mammalian MsrB2 and MsrB3 in *Saccharomyces cerevisiae*, and determined that their heterologous expression could protect yeast cells against oxidative stress-induced cell death, similar to the

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protective effects of MsrA overexpression.

RESULTS

Overexpression of mammalian Msr genes in *S. cerevisiae*

In order to determine whether heterologous overexpression of the mammalian Msr genes in yeast cells confers resistance against oxidative stress, we selected mouse MsrA and MsrB2, as well as human MsrB3A and MsrB3B, for this study. The selenoprotein, MsrB1, was excluded because yeast cells do not encode selenocysteine biosynthesis and incorporation systems. We separately transformed the constructs harboring the Msr genes into wild-type *S. cerevisiae* cells (GY1), and conducted Western blot analyses and enzyme assays to verify their expression. For Western blots, the whole cell extracts were prepared via NaOH treatment, as described in the Materials and Methods section. As shown in Fig. 1A, the MsrA, MsrB2, and MsrB3B proteins were detected, whereas the MsrB3A was not detected in the whole cell extracts.

The alternatively spliced variants of MsrB3A and MsrB3B differ only in their N-terminal region signal peptides. The MsrB3A harbors an ER signal peptide, whereas the MsrB3B harbors a mitochondrial signal peptide. Both proteins contain an ER-retention signal KAEL sequence at their C-terminal sections. The ER localization of MsrB3A protein is dependent on this KAEL retention signal in mammalian cells. However, there remained the possibility that this ER-retention signal would not function in yeast cells. In order to assess this possibility, we attempted to determine whether the MsrB3A protein was secreted extracellularly. A band corresponding to MsrB3 was observed on the Western blot assay of the culture broth (data not shown). Additionally, this culture broth evidenced a 2-fold increase in MsrB activity when compared to that observed in the control cells (data not shown). Thus, these results indicate that the mammalian ER-retention signal (KAEL) of MsrB3A does not function as a retention signal in yeast cells.

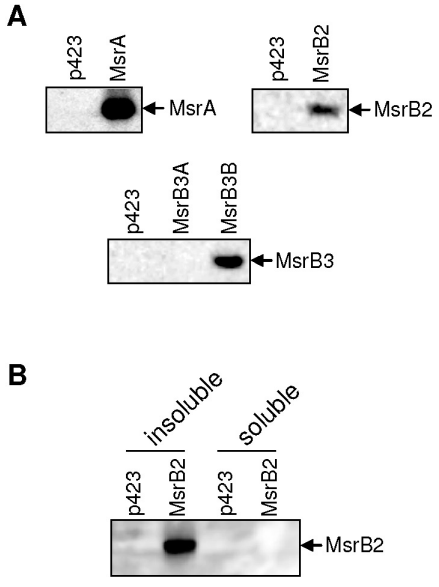

Fig. 1. Overexpression of mammalian MsrA and MsrB genes in wild-type *S. cerevisiae* (GY1). (A) Western blots using whole cell extracts. (B) Western blot of the soluble and insoluble fractions from cell lysates. The MsrB2 protein was exclusively detected in the insoluble fraction.

We then assessed the enzyme activities of the GY1 cells overexpressing MsrA, MsrB2, or MsrB3B using crude extracts (soluble fractions of the lysates) (Table 1). As expected, the crude extract from the MsrA-overexpressing cells evidenced a 60-fold increase in MsrA activity compared to that observed from the control cells. However, the MsrB2 crude extract evidenced no increase in MsrB activity, whereas the MsrB3B crude extract evidenced a 40-fold increase in MsrB activity when compared to the controls. Consistent with the results of the enzyme assays, Western blot analysis revealed that the MsrB2 protein was not detected in the crude extract (soluble fraction), but was present in the insoluble fraction of the lysate (Fig. 1B).

Subcellular localization of MsrB2 and MsrB3B in yeast cells

Mouse MsrA containing a mitochondrial signal peptide is known to be located in both the cytosol and mitochondria when expressed in *S. cerevisiae* (7), similar to its cellular localization in mammalian cells. As previously mentioned, both MsrB2 and MsrB3B harbor N-terminal mitochondrial signal peptides and are targeted to the mitochondria in mammalian cells. To assess the subcellular localization of these proteins in yeast cells, we isolated the cytosolic and mitochondrial fractions from the GY1 cells overexpressing MsrB2 or MsrB3B. As shown in Fig. 2A, the majority of MsrB2 was localized in the mitochondrial fraction, although this protein was also detected at lower levels in the cytosolic fraction. A slight increase in

Table 1. Enzyme activities of yeast cells overexpressing Msr genes

Strains	Forms	Specific activity (pmol/min/mg crude protein)	
		MsrA	MsrB
GY1 (wild-type)	p423	14	7
	MsrA	820	NA
	MsrB2	NA	10
	MsrB3B	NA	270
GY2 (*msrB* mutant)	p423	NA	10 ± 2
	MsrB2	NA	15 ± 3
	MsrB3B	NA	430
GY4 (*msrA* mutant)	p423	8	NA
	MsrA	680	NA

Cells were grown aerobically at 30°C for 20-24 h in YNBD media. The supernatant of lysates (200 μg crude protein) was used for the enzyme assays. NA, not assayed.

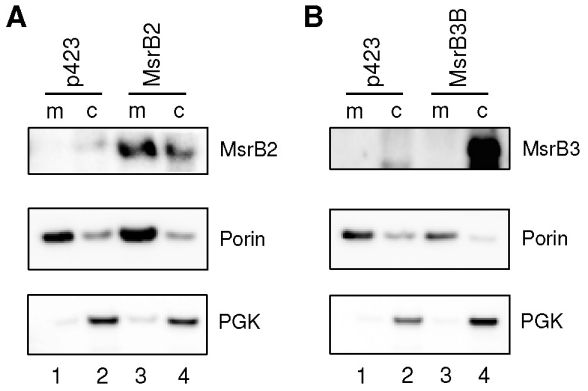

Fig. 2. Subcellular localization of MsrB2 and MsrB3B in *S. cerevisiae*. Mitochondrial and cytosolic fractions were isolated from GY1 cells expressing MsrB2 (A) or MsrB3B (B). A total of 2.5 μg of mitochondrial and 5 μg of cytosolic proteins were subjected to SDS-PAGE and Western blotting. Porin and 3-phosphoglycerate kinase (PGK) were used as mitochondrial and cytosolic markers, respectively. The mitochondrial and cytosolic fractions of all samples demonstrated slight contamination with cytosol and mitochondria, respectively. (A) MsrB2 localization: Lanes 1 and 2, mitochondrial (m) and cytosolic (c) fractions from cells containing the p423 vector, respectively; lanes 3 and 4, mitochondrial and cytosolic fractions from cells expressing MsrB2, respectively. (B) MsrB3B localization: Lane 1 and 2, the same samples of lanes 1 and 2 in (A), respectively; lanes 3 and 4, mitochondrial and cytosolic fractions from cells expressing MsrB3B, respectively.

MsrB activity was noted in this mitochondrial fraction when compared with the activity observed in the mitochondrial fraction of the control cells (Supplementary Fig. S1). Interestingly, the MsrB3B protein was localized in the cytosol, but not in the mitochondrial fraction (Fig. 2B). These results suggest that the MsrB2 mitochondrial signal peptide (MARLLRALRGLPLLQAP GRLARG) is functional in yeast cells, whereas the MsrB3B mitochondrial targeting peptide (MSAFNLLHLVTKSQPVALRACG LP) is not.

Overexpression of MsrB2, MsrB3, or MsrA protects yeast cells against oxidative stress-induced cell death

Next, we assessed the role of the MsrB2, MsrB3B, and MsrA in cellular protection against H_2O_2-induced oxidative stress. When the wild-type GY1 cells overexpressing the Msr genes were treated with 2 mM H_2O_2 at 12 h culture time, the MsrA- and MsrB2-overexpressing cells were more resistant to the H_2O_2 treatment (Supplementary Fig. S2). However, the overexpression of MsrB3B was shown to have no effect on protection against H_2O_2-mediated cell death. Similar results were observed in plate assays when treated with 2 mM H_2O_2 (Supplementary Fig. S2).

We further analyzed the antioxidant role of MsrA and MsrB proteins using *msrA* and *msrB* deletion mutant strains, respectively. It should be noted that deletions of *msrA* and *msrB* render yeast cells more sensitive to H_2O_2-induced oxidative stress (19, 20). As shown in Fig. 3A, MsrA overexpression

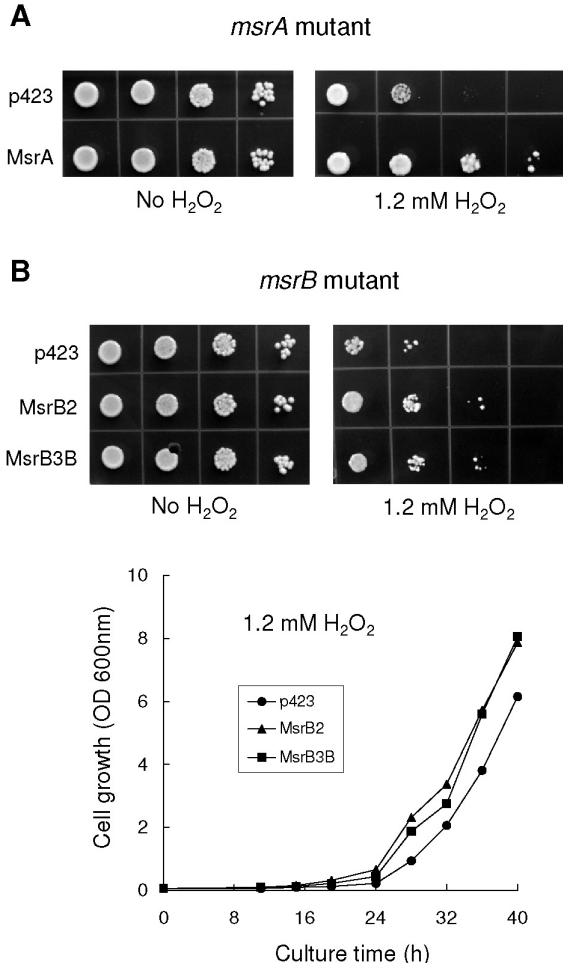

Fig. 3. Overexpression of MsrB3, MsrB2, or MsrA protects against oxidative stress-induced cell death. (A) Overexpression of *MsrA* in *msrA* mutant GY4 cells. The cells were incubated on YNBD agar medium with no treatment (left) or 1.2 mM H_2O_2 (right) at 30°C for 3 days. (B) Overexpression of MsrB2 and MsrB3B in *msr*B mutant GY2 cells. Plate (upper panel) and liquid assays (lower panel) were performed. For the plate assays, the GY2 cells expressing *MsrBs* were incubated on YNBD agar medium in the absence (left) or presence of 1.2 mM H_2O_2 (right) at 30°C for 3 days. For the liquid assay, the cells were cultured in YNBD media with 1.2 mM H_2O_2 at 30°C. All data in these figures are representative of at least two independent experiments.

in the *msrA* mutant GY4 cells conferred a significant level of resistance to H_2O_2-mediated oxidative stress, relative to the resistance observed in the wild-type cells. The overexpression of MsrA was confirmed via enzyme assay (80-fold increased MsrA activity, Table 1) and Western blotting (data not shown). As expected, the overexpression of MsrB2 in the *msrB* mutant GY2 cells effected a profound protective effect against H_2O_2-mediated cell death (Fig. 3B, upper panel). Importantly, MsrB3B overexpression in these *msrB* mutant cells clearly protected them against H_2O_2-mediated cell death. The antioxidant

role of MsrB3B was further verified in liquid cultures (Fig. 3B, lower panel).

Western blot analysis using whole cell extracts revealed that both MsrB2 and MsrB3B were expressed in the *msrB* mutant GY2 cells (data not shown). We then assessed the enzyme activities of these cells overexpressing MsrB2 or MsrB3B (Table 1). The crude extract of the MsrB3B-overexpressing cells evidenced a 40-fold increase in MsrB activity when compared to that of the control cells. However, the MsrB2 crude extract showed no increase in MsrB activity, similar to the absence of increased activity in the crude extract of the wild-type GY1 cells overexpressing this protein. We determined that this protein was detected in the insoluble fraction of the lysates, but not in the crude extract (data not shown), as was shown in the MsrB2-overexpressing GY1 cells.

In conclusion, the overexpression of mammalian MsrB2 and MsrB3 could protect yeast cells against H_2O_2-mediated cell death, as could the overexpression of mammalian MsrA.

DISCUSSION

The Msr genes encode important oxidized protein repair enzymes. This gene family includes the MsrA and MsrB proteins, which catalyze the reduction of *S*- and *R*-forms of methionine sulfoxide back to methionine residues within proteins, respectively. Both the MsrA and MsrB proteins are necessary for the maintenance of the methionine sulfoxide reduction system in cells. The functions of MsrA in antioxidant defense and protein maintenance, as well as its functions in the aging process and neurodegenerative diseases, have been studied extensively. In contrast, the function of MsrB in these biological and pathological processes remains poorly understood. In the current study, we demonstrated that the heterologous overexpression of mammalian MsrB2, MsrB3, or MsrA protects yeast cells against H_2O_2-mediated cell death. Recently, while this study was prepared for publication, a study by Cabreiro *et al*. (18) reported that the overexpression of MsrB2 in T-lymphoblastic leukemia MOLT-4 cells protects against H_2O_2-mediated oxidative stress. Consistent with their results, the overexpression of MsrB2 in lower eukaryotic yeast cells increased cell survival against oxidative stress. In addition, this study provides the first evidence that the overexpression of the MsrB3 isozyme also protects against oxidative stress-induced cell death. The homologous overexpression of MsrA in *S. cerevisiae* has previously been reported to protect against H_2O_2-mediated oxidative stress (12). Our present study assaying the heterologous overexpression of MsrA and MsrB genes in yeast cells demonstrated that the mammalian MsrA and MsrB enzymes perform pivotal functions in cellular protection against oxidative stress, and is indicative of broad substrate specificity, due to the fact that they function in both mammalian and yeast cells.

The mitochondrial MsrB2 protein was shown to be localized in the mitochondria of *S. cerevisiae*. However, the mitochondrial MsrB3B was not targeted to the mitochondria, but was located in the cytosol of *S. cerevisiae*. The N-terminal peptide sequence of MsrB2 (MARLLRALRGLPLLQAPGRLARG) was predicted by the MitoProt program to be a typical mitochondrial targeting sequence (MTS), and has been calculated to form an amphipathic helix. However, the MsrB3B mitochondrial signal peptide (MSAFNLLHLVTKSQPVALRACGLP) was not predicted by this program to be a typical MTS. In mammalian cells, this signal peptide is functional for the targeting of MsrB3B to the mitochondria, but in *S. cerevisiae* it is not functional in mitochondrial targeting, and this may be due to non-recognition by a cytosolic import stimulation factor expressed in yeast cells (21). Furthermore, the ER-retention signal (KAEL) of MsrB3A did not function as a retention signal in *S. cerevisiae*. The optimal ER-retention signal in *S. cerevisiae* is known to be the tetrapeptide HDEL, whereas in animal cells the KDEL sequence functions best (22).

It should be noted that MsrB2 is a soluble protein when expressed in *Escherichia coli*. Interestingly, this protein was detected exclusively in the insoluble fraction of the yeast cell lysates, and was shown to be largely localized to the mitochondrial fraction. The soluble fractions of the cell lysates harbored a significant quantity of porin protein (a mitochondrial marker), indicating that the mitochondria were largely broken during the preparation of the cell extracts (Supplementary Fig. S3). Additionally, the porin protein was detected in the insoluble fractions. Thus, these observations indicate that the MsrB2 protein may be bound to the mitochondrial membrane, and thus precipitated in the pellet of the cell lysate.

MATERIALS AND METHODS

Yeast strains and constructs

S. cerevisiae wild-type strain BY4741 (*MATa his3 leu2 met15 ura3*) (GY1), *msrB* mutant GY2 (*MATa his3 leu2 met15 ura3 ΔmsrB::KAN*), and *msrA* mutant GY4 (*MATa his3 leu2 met15 ura3 ΔmsrA::URA3*) were used (19). The constructs harboring mammalian Msr genes are as follows: p423-MsrA for mouse MsrA (7), p423-MsrB2 for mouse MsrB2, p423-MsrB3A for human MsrB3A, and p423-MsrB3B for human MsrB3B (9). All of these constructs encode full-length Msr proteins and are expressed from the yeast p423GPD vector (23).

Transformation and growth of yeast cells

All constructs were transformed into yeast cells via the lithium acetate method and transformants were selected for histidine prototrophy. Cells containing the p423 vector or plasmids expressing the Msr genes were aerobically grown at 30°C in yeast nitrogen base minimal medium supplemented with 2% glucose (YNBD).

Cell viability following H_2O_2 treatments

Overnight cultures of yeast cells containing each construct were diluted to an optical density (OD) of 10 at 600 nm with

YNBD medium, and 0.5 ml of the adjusted cultures was added to 50 ml of YNBD media in a 250 ml flask. The cultures were treated with the indicated concentrations of H_2O_2, either at the initiation of growth or during growth. The cells were aerobically grown at 30°C with shaking, and the growth was measured by OD at 600 nm.

For plate assays, the overnight cultures were each adjusted to an OD of 2.5, 0.25, 0.025, and 0.0025 via serial dilution. Each diluted sample (5 µl) was spotted onto YNBD agar medium in the presence or absence of the indicated concentrations of H_2O_2. Cell growth was examined after 3-4 days of incubation at 30°C.

Isolation of mitochondria from yeast cells expressing MsrB2 or MsrB3B

Wild-type GY1 cells containing the p423, p423-MsrB2, or p423-MsrB3B were grown aerobically at 30°C for 40 h in YNBD media. Mitochondria were isolated as previously described (7). The purity of the isolated mitochondrial and cytosolic fractions was analyzed via Western blot using anti-porin and anti-3-phosphoglycerate kinase antibodies (Molecular Probes) as the mitochondrial and cytosolic markers, respectively.

Preparation of cell extracts

Whole cell extracts were prepared via alkali treatment and boiling, as previously described (24). In brief, yeast cells were harvested by centrifugation from liquid cultures, and the cell pellets were resuspended in 0.2 M NaOH and incubated for 5 min at room temperature. After centrifugation, the cells were resuspended in SDS sample loading buffer and boiled for 5 min. The supernatant was then subjected to SDS-PAGE for Western blot analysis.

For enzyme assays, yeast cells were harvested by centrifugation, resuspended in PBS buffer, and lysed using glass beads (BeadBeater; BioSpec Products). The lysates were subjected to 20 min of centrifugation at 15,000 rpm. The supernatant was utilized as a crude extract for enzyme assays, and the pellet was employed as the insoluble lysate fraction.

Enzyme assays

MsrA and MsrB activities were measured in the presence of dithiothreitol (DTT). The reaction mixture (100 µl) contained 50 mM sodium phosphate (pH 7.5), 50 mM NaCl, 20 mM DTT, 200 µM dabsyl-methionine-*S*-sulfoxide (for MsrA) or dabsyl-methionine-*R*-sulfoxide (for MsrB), and 200 µg crude protein. The reactions were conducted for 30 min at 37°C and the reaction product, dabsyl-Met, was analyzed via HPLC as previously described (25).

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REFERENCES

1. Weissbach, H., Etienne, F., Hoshi, T., Heinemann, S. H., Lowther, W. T., Matthews, B., St John, G., Nathan, C. and Brot, N. (2002) Peptide methionine sulfoxide reductase: structure, mechanism of action, and biological function. *Arch. Biochem. Biophys*. **397**, 172-178.
2. Kim, H. Y. and Gladyshev, V. N. (2007) Methionine sulfoxide reductases: selenoprotein forms and roles in antioxidant protein repair in mammals. *Biochem. J*. **407**, 321-329.
3. Moskovitz, J. (2005) Methionine sulfoxide reductases: ubiquitous enzymes involved in antioxidant defense, protein regulation, and prevention of aging-associated diseases. *Biochim. Biophys. Acta*. **1703**, 213-219.
4. Haenold, R., Wassef, R., Hansel, A., Heinemann, S. H. and Hoshi, T. (2007) Identification of a new functional splice variant of the enzyme methionine sulphoxide reductase A (MSRA) expressed in rat vascular smooth muscle cells. *Free Radic. Res*. **41**, 1233-1245.
5. Kim, H. Y. and Gladyshev, V. N. (2006) Alternative first exon splicing regulates subcellular distribution of methionine sulfoxide reductases. *BMC Mol. Biol*. **7**, 11.
6. Lee, J. W., Gordiyenko, N. V., Marchetti, M., Tserentsoodol, N., Sagher, D., Alam, S., Weissbach, H., Kantorow, M. and Rodriguez, I. R. (2006) Gene structure, localization and role in oxidative stress of methionine sulfoxide reductase A (MSRA) in the monkey retina. *Exp. Eye Res*. **82**, 816-827.
7. Kim, H. Y. and Gladyshev, V. N. (2005) Role of structural and functional elements of mouse methionine-S-sulfoxide reductase in its subcellular distribution. *Biochemistry* **44**, 8059-8067.
8. Vougier, S., Mary, J. and Friguet, B. (2003) Subcellular localization of methionine sulphoxide reductase A (MsrA): evidence for mitochondrial and cytosolic isoforms in rat liver cells. *Biochem. J*. **373**, 531-537.
9. Kim, H. Y. and Gladyshev, V. N. (2004) Methionine sulfoxide reduction in mammals: characterization of methionine-*R*-sulfoxide reductases. *Mol. Biol. Cell* **15**, 1055-1064.
10. Hansel, A., Heinemann, S. H. and Hoshi, T. (2005) Heterogeneity and function of mammalian MSRs: enzymes for repair, protection and regulation. *Biochim. Biophys. Acta*. **1703**, 239-247.
11. Kim, H. Y. and Gladyshev, V. N. (2004) Characterization of mouse endoplasmic reticulum methionine-*R*-sulfoxide reductase. *Biochem. Biophys. Res. Commun*. **320**, 1277-1283.
12. Moskovitz, J., Flescher, E., Berlett, B. S., Azare, J., Poston, J. M. and Stadtman, E. R. (1998) Overexpression of peptide-methionine sulfoxide reductase in *Saccharomyces cerevisiae* and human T cells provides them with high resistance to oxidative stress. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 14071-14075.
13. Kantorow, M., Hawse, J. R., Cowell, T. L., Benhamed, S., Pizarro, G. O., Reddy, V. N. and Hejtmancik, J. F. (2004) Methionine sulfoxide reductase A is important for lens cell viability and resistance to oxidative stress. *Proc. Natl.*

Acad. Sci. U.S.A. **101**, 9654-9659.
14. Picot, C. R., Petropoulos, I., Perichon, M., Moreau, M., Nizard, C. and Friguet, B. (2005) Overexpression of MsrA protects WI-38 SV40 human fibroblasts against H_2O_2-mediated oxidative stress. *Free Radic. Biol. Med.* **39**, 1332-1341.
15. Yermolaieva, O., Xu, R., Schinstock, C., Brot, N., Weissbach, H., Heinemann, S. H. and Hoshi, T. (2004) Methionine sulfoxide reductase A protects neuronal cells against brief hypoxia/reoxygenation. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 1159-1164.
16. Prentice, H. M., Moench, I. A., Rickaway, Z. T., Dougherty, C. J., Webster, K. A. and Weissbach, H. (2008) MsrA protects cardiac myocytes against hypoxia/reoxygenation induced cell death. *Biochem. Biophys. Res. Commun.* **366**, 775-778.
17. Marchetti, M. A., Lee, W., Cowell, T. L., Wells, T. M., Weissbach, H. and Kantorow, M. (2006) Silencing of the methionine sulfoxide reductase A gene results in loss of mitochondrial membrane potential and increased ROS production in human lens cells. *Exp. Eye Res.* **83**, 1281-1286.
18. Cabreiro, F., Picot, C. R., Perichon, M., Castel, J., Friguet, B. and Petropoulos, I. (2008) Overexpression of mitochondrial methionine sulfoxide reductase B2 protects leukemia cells from oxidative stress-induced cell death and protein damage. *J. Biol. Chem.* **283**, 16673-16681.
19. Kryukov, G. V., Kumar, R. A., Koc, A., Sun, Z. and Gladyshev, V. N. (2002) Selenoprotein R is a zinc-containing stereo-specific methionine sulfoxide reductase. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 4245-4250.
20. Moskovitz, J., Berlett, B. S., Poston, J. M. and Stadtman, E. R. (1997) The yeast peptide-methionine sulfoxide reductase functions as an antioxidant *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9585-9589.
21. Omura, T. (1998) Mitochondria-targeting sequence, a multi-role sorting sequence recognized at all steps of protein import into mitochondria. *J. Biochem.* **123**, 1010-1016.
22. Lewis, M. J., Sweet, D. J. and Pelham, H. R. (1990) The ERD2 gene determines the specificity of the luminal ER protein retention system. *Cell* **61**, 1359-1363.
23. Mumberg, D., Muller, R. and Funk, M. (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* **156**, 119-122.
24. Kushnirov, V. V. (2000) Rapid and reliable protein extraction from yeast. *Yeast* **16**, 857-860.
25. Kumar, R. A., Koc, A., Cerny, R. L. and Gladyshev, V. N. (2002) Reaction mechanism, evolutionary analysis, and role of zinc in Drosophila methionine-R-sulfoxide reductase. *J. Biol. Chem.* **277**, 37527-37535.