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Chromosomal localization of the mammalian peptide-methionine sulfoxide reductase gene and its differential expression in various tissues

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ABSTRACT **Peptide methionine sulfoxide reductase (MsrA; EC 1.8.4.6) is a ubiquitous protein that can reduce methionine sulfoxide residues in proteins as well as in a large number of methyl sulfoxide compounds. The expression of MsrA in various rat tissues was determined by using immunocytochemical staining. Although the protein was found in all tissues examined, it was specifically localized to renal medulla and retinal pigmented epithelial cells, and it was prominent in neurons and throughout the nervous system. In addition, blood and alveolar macrophages showed high expression of the enzyme. The *msrA* gene was mapped to the central region of mouse chromosome 14, in a region of homology with human chromosomes 13 and 8p21.**

Oxidation of methionine residues in proteins is mediated by various biological oxidants such as H_2O_2, hydroxyl radicals, hypochlorite, and superoxide ions (1). The oxidized product, methionine sulfoxide [Met(O)] can be enzymatically reduced back to methionine by peptide methionine sulfoxide reductase (MsrA; EC 1.8.4.6), which has been detected in virtually all organisms and tissues examined (2–4).

The *Escherichia coli* gene and a mammalian *msrA* cDNA have been cloned and sequenced (5, 6). In addition, an *E. coli msrA* mutant has recently been shown to be more sensitive to oxidative damage than the parent strain (7). The mammalian *msrA* cDNA encodes a protein that has a high similarity to the *E. coli* MsrA (61% identity) and in general, the characteristics of the two enzymes are similar (6). *msrA* mRNA is highly expressed in rat and human kidney, but to a lesser extent in other rat tissues (6). The highest specific activity of the mammalian MsrA enzyme was found in cell-free extracts prepared from rat kidney (6) and human neutrophils (8). Neutrophils and macrophages are known to produce reactive oxygen species during a respiratory burst that kill invading bacteria during phagocytosis (9). It has been suggested that MsrA functions to repair oxidative damage to proteins that may occur during an oxidative burst or other situations where reactive oxygen radicals exist (7).

In this study we describe the chromosomal localization of the *msrA* gene in the mouse and MsrA distribution in various rat tissues.

MATERIALS AND METHODS

Interspecific Backcross Mapping. Interspecific backcross progeny were generated by mating (C57BL/6J × *Mus spretus*)F_1 females and C57BL/6J males as described (10). A total of 205 backcross mice were used to map the *msrA* locus (see text for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed as described (11). All blots were prepared using Hybond-N^+ nylon membrane (Amersham). The probe, a 228-bp PCR-generated mouse cDNA fragment (6), was labeled with [α-^{32}P]dCTP by random priming (Stratagene), and the blots were washed at 65°C in a buffer containing 0.8× SSCP and 0.1% SDS (1× SSCP = 120 mM NaCl/15 mM sodium citrate/20 mM sodium phosphate, pH 6.8). A 5.8-kb fragment was detected in *Sph* I-digested C57BL/6J (B) genomic DNA and an 8.2-kb fragment, in *Sph* I-digested *M. spretus* (S) DNA. Likewise, 6.9- and 8.4-kb fragments were detected in *Bgl* II-digested B and S DNA, respectively. The presence or absence of the 8.2-kb *M. spretus*-specific *Sph* I fragment and the 8.4-kb *M. spretus*-specific *Bgl* II fragment, which cosegregated, was followed in backcross mice. The two sets of data were combined in determining the map location of the mouse *msrA*.

A description of the probes and restriction fragment length polymorphisms (RFLPs) for loci linked to *msrA*, including the gap junction membrane channel protein β-2 (Gjb2), neurofilament light polypeptide (Nfl), and surfactant-associated protein 2 (Sftp2), have been previously reported (12–14). Recombination distances were calculated as described by Green (15), using the computer program SPRETUS MADNESS. Gene order was determined by minimizing the number of double and multiple recombination events across the chromosome.

Immunocytochemistry. Adult female rats (60 days old) were purchased from Charles River Breeding Laboratories. The rats were deeply anesthetized with a mixture of ketamine (80 mg/ml, Sigma) and xylazine (10 mg/ml, Sigma) in physiological saline and perfused intracardially with phosphate-buffered saline (PBS); 10 mM sodium phosphate, pH 7.2/130 mM NaCl) followed by 4% paraformaldehyde in PBS. Brain, retina, kidney, and lungs were postfixed for 1 h in the same fixative at room temperature and cryoprotected in 30% sucrose in PBS for 2 days at 4°C. Macrophages were obtained from mouse peritoneum and were processed in a similar fashion. Free-floating 60-μm tissue sections were cut, soaked in PBS, and treated with 70% (vol/vol) methanol and 0.3% hydrogen peroxide for 20 min. Following blocking with 0.5 M Tris·HCl, pH 7.6, containing 5% goat serum and 0.5% Triton X-100 for 1 h, the sections were incubated with anti-MsrA rabbit antibodies (diluted 1:100) for 6 h at room temperature. Rabbit antibodies were raised against the recombinant bovine MsrA (6) and were affinity-purified on an Affi-Gel 10-bovine MsrA column, according to the procedure described by Bio-Rad. The specificity of the antibodies was confirmed by Western blot analysis, which showed one 26-kDa protein band from bovine and rat tissue extracts. Tissue sections were then incubated for 2 h at room temperature with a biotinylated antibody to rabbit

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Abbreviations: Met(O), methionine sulfoxide; RFLP, restriction fragment length polymorphism; RPE, retinal pigmented epithelium.

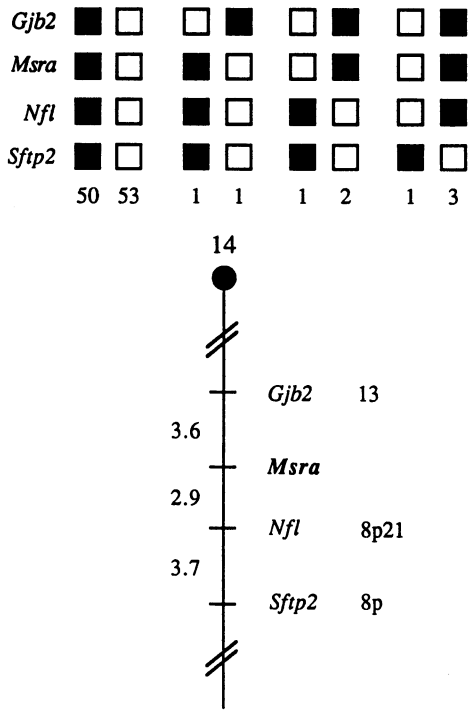

FIG. 1. *msrA* maps in the middle region of mouse chromosome 14. *msrA* was mapped to mouse chromosome 14 by interspecific backcross analysis. The segregation patterns of *msrA* and flanking genes in 112 backcross animals are shown at the top. For some individual pairs of loci, more than 112 animals were typed (see text). Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*)F_1 parent. The black boxes represent the presence of a C57BL/6J allele, while the white boxes represent the presence of an *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial chromosome 14 linkage map showing the location of *msrA* in relation to linked genes is shown at the bottom. Recombination distances between loci in cM are shown to the left of the chromosome and the positions of loci in human chromosomes, where indicated, are shown to the right. References for human map positions can be obtained from GDB (Genome Data Base), a computerized data base of human linkage information maintained by the William H. Welch Medical Library of The Johns Hopkins University (Baltimore).

IgG followed by incubation with streptavidin coupled to horseradish peroxidase (Vector Laboratories) in the same buffer without Triton X-100. The sections were washed three times for 10 min each time in 0.5 M Tris·HCl, pH 7.6, and were finally developed with a diaminobenzidine (DAB) reagent substrate kit (Vector Laboratories).

RESULTS AND DISCUSSION

Chromosomal Localization. The location in the mouse genome of the homolog to the mammalian *msrA* was determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J × *M. spretus*)F_1 × C57BL/6J] mice. This interspecific backcross mapping panel has been typed for over 1900 loci that are well distributed among all mouse autosomes and the X chromosome (ref. 10; N.G.C. and N.A.J., unpublished results). C57BL/6J and *M. spretus* DNAs were digested with several restriction enzymes and analyzed by Southern blot hybridization for informative RFLPs by using a mouse *msrA* cDNA probe (see *Materials and Methods*). An *M. spretus*-specific *Sph* I and an *M. spretus*-specific *Bgl* II RFLP, which cosegregated, were used to follow the segregation of the *msrA* locus in backcross DNAs. The mapping results indicated that *msrA* is located in the middle region of mouse chromosome 14 (Fig. 1). Although at least 112 mice were analyzed for every marker shown in the haplotype analysis (Fig. 1), up to 162 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies, using the combined data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci (in parentheses) and the most likely gene order were centromere–*Gjb2* (5/140)–*msrA* (4/137)–*Nfl* (6/162)–*Sftp2*. The recombination frequencies [expressed in parentheses as genetic distances in centimorgans (cM) ± the standard error] were centromere–*Gjb2* (3.6 ± 1.6)–*msrA* (2.9 ± 1.4)–*Nfl* (3.7 ± 1.5)–*Sftp2*. The human homolog of *msrA* has not been mapped in humans, but *msrA* should map on either human chromosome 13 or 8p21 on the basis of homology of these human chromosomes to the region on mouse chromosome 14 (Fig. 1).

Expression of MsrA *in Situ*. Using immunocytochemical techniques and purified antibodies against bovine recombinant MsrA, we have investigated the expression of the enzyme in various rat and mouse tissues. Specific immunostaining was found in cells of liver, lung, heart, retina, kidney, brain, and macrophages. No staining was detected when the antibodies were preincubated with purified MsrA. These results corroborated earlier enzymatic data that showed the ubiquitous nature of this protein (3). Although in liver, lung, and heart diffuse staining was detected (except for the alveolar macrophages residing in the lung) the other four tissues examined showed a more specific localization pattern. In the retina (Fig. 2) some staining was detected in the inner segments as well as in the inner nuclear layer, but the most intense staining was

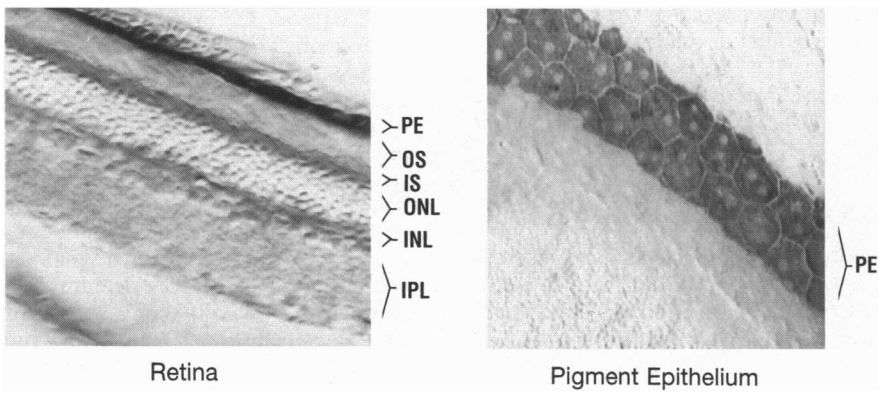

FIG. 2. Localization of MsrA in rat retina. Immunocytochemistry was performed as described in the text. (*Left*) ×240 magnification of the retina. (*Right*) ×560 magnification of pigment epithelium cells. PE, pigment epithelium; OS, outer segments; IS, inner segments; ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer.

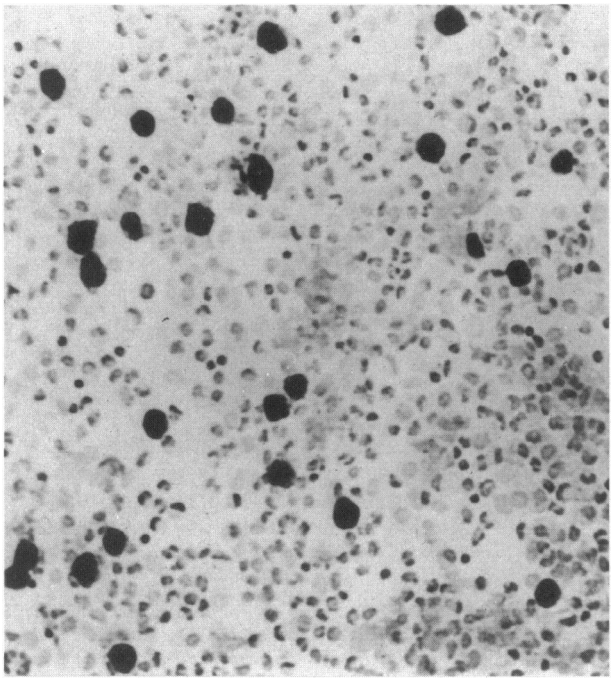

FIG. 3. Mouse peritoneal macrophages stained with antibodies against MsrA. (×240.)

found in the pigmented epithelium (RPE) cells which have a distinctive shape, appearing as hexagonal prisms (Fig. 2). Prominent staining was also observed in mouse peritoneal macrophages (Fig. 3) as well as alveolar macrophages (data not shown).

The finding of high levels of MsrA in RPE cells was of special interest. One of the important functions of these cells, which are located between the sensory retina and the choroid, is the continuous phagocytosis and degradation of rod and cone outer segments (ROS) (16). The RPE has the highest catalase activity of any ocular tissue measured and contains high concentrations of antioxidants, which seem to be essential for protecting the RPE cells against oxidative damage (17–21). Other evidence suggests that the normal process of phagocytosis of ROS by the RPE cells may be an oxidative stress to the RPE (22). Thus, both macrophages and RPE cells have, in common, the abilities to phagocytize and to produce oxidants (8, 9), and they contain high levels of MsrA. A defect in the function of RPE cells may lead to the degeneration of the sensory retina (23) and may contribute to age-related maculopathy and retinitis pigmentosa (RP) in humans (23). RP is a set of hereditary retinal diseases in which photoreceptor cells degenerate (23). Over 20 loci have been mapped in which mutations cause RP, and one of them is linked to chromosome 8p11–q21 (24). Further studies are required to determine the

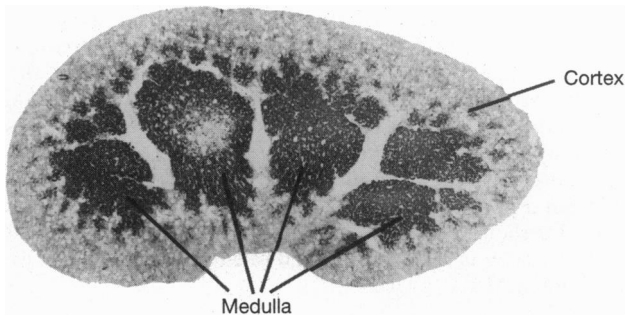

FIG. 4. Localization of MsrA in rat kidney. (×200.)

role of MsrA in these cells and if the *msrA* gene is involved in RP. A general role of MsrA could be to provide a mechanism to ensure that Met(O) residues in proteins do not accumulate as a result of oxidative damage.

Staining was localized specifically to the cells of the kidney medulla, where the loop of Henle and the collecting tubules are found (Fig. 4). No significant difference in staining between the inner medulla and outer medulla was observed. Also, in rabbit liver and kidney microsomes, methionine is the first identified endogenous compound metabolized to diastereomeric sulfoxides by flavin-containing monooxygenases (25). A possible role of MsrA in kidney may be to help salvage free methionine and protect proteins from inactivation due to increased methionine oxidation.

Sagittal sections of the adult rat brain revealed that methionine sulfoxide reductase-like immunoreactivity (MSRLI) was much more pronounced in neurons than in glia (Fig. 5). Furthermore, some populations of neurons expressed the enzyme at much higher levels than others. For example, relatively high levels of MSRLI were detected in cerebellum, where it appeared to be associated with granule and Purkinje cells as well as deeper nuclear neurons. In general, immunostaining was seen in the cell soma and dendrites, while little MSRLI was detected in axons of cerebellar neurons. In addition to the cerebellum, elevated immunoreactivity was seen in the olfactory bulb, the substantia nigra, hippocampal CA1 neurons, the pontine reticular nucleus, and large neurons located in the spinal cord, medulla oblongata, and cerebral cortex.

This distribution of immunostaining did not appear to correlate with any particular neurotransmitter or functional system in the brain. However, the preponderance of the enzyme in neurons suggests that the expression profile may be reflective of enhanced demand for reductase function in neurons. Given that neurons have very high metabolic rates that lead to oxidative environments, particularly during periods of hyperstimulation or hypoxia/ischemia, one determinant of enzyme expression may be the intrinsic firing rates of particular neurons (26). This being the case, this enzyme may

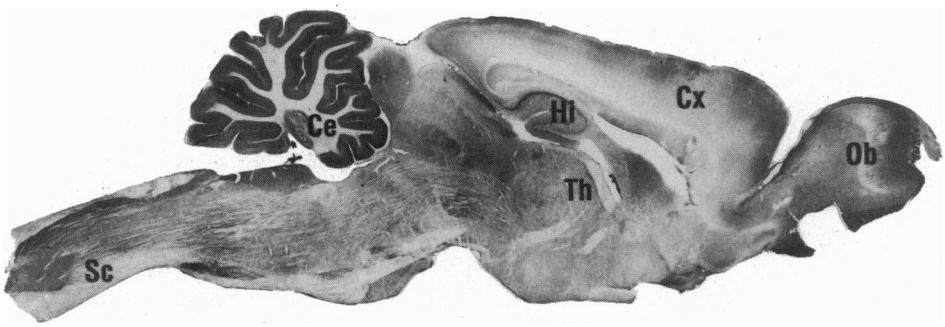

FIG. 5. Saggital section of rat brain stained with antibodies against MsrA. (×240.) CE, cerebellum; Cx, cerebral cortex; Hi, hippocampus; Ob, olfactory bulb; Sc, spinal cord; Th, thalamus.

contribute to the resistance of neurons to oxidative damage in both normal and pathological circumstances such as stroke. In addition, there is an increasing amount of evidence that oxidative stress is a causal, or at least an ancillary, factor in the neuropathology of several adult neurodegenerative disorders, as well as in stroke, trauma, and seizures (27).

It remains unclear whether MsrA activity is required to maintain a basal protection against protein oxidation in the brain. Further experiments, such as gene "knock-out" and overexpression in mice, are needed to elucidate the role of the mammalian MsrA *in vivo*.

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