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Methionine sulfoxide reductase contributes to meeting dietary methionine requirements

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ABSTRACT

Methionine sulfoxide reductases are present in all aerobic organisms. They contribute to antioxidant defenses by reducing methionine sulfoxide in proteins back to methionine. However, the actual *in vivo* roles of these reductases are not well defined. Since methionine is an essential amino acid in mammals, we hypothesized that methionine sulfoxide reductases may provide a portion of the dietary methionine requirement by recycling methionine sulfoxide. We used a classical bioassay, the growth of weanling mice fed diets varying in methionine, and applied it to mice genetically engineered to alter the levels of methionine sulfoxide reductase A or B1. Mice of all genotypes were growth retarded when raised on chow containing 0.10% methionine instead of the standard 0.45% methionine. Retardation was significantly greater in knockout mice lacking both reductases. We conclude that the methionine sulfoxide reductases can provide methionine for growth in mice with limited intake of methionine, such as may occur in the wild.

Introduction

Virtually all organisms from bacteria to mammals have several methionine sulfoxide reductases (Msr)[1] that catalyze the reduction of methionine sulfoxide back to methionine. The oxidation of methionine to its sulfoxide can be effected by reactive oxygen and nitrogen species. Approximately 98% of methionine in organisms is in proteins, and the steady state content of methionine sulfoxide in mouse tissues is 4–10% of the total methionine [1,2]. Recovery of this oxidized methionine could therefore contribute to meeting the nutritional requirement for methionine (Fig. 1).

The oxidation of methionine produces two stereospecific forms of methionine sulfoxide. The R-form is reduced specifically by MsrB and the S-form by MsrA. In mammals, there is only one form of MsrA while there are three isoforms of MsrB: MsrB1, MsrB2 and MsrB3, each encoded by a different gene. MsrB1 is the most abundant of the B isozymes and is found in the cytosol and the nucleus. There is considerable evidence that MsrA and MsrB provide an antioxidant defense by scavenging reactive oxygen species through cyclic oxidation and reduction of methionine and methionine sulfoxide [3–12]. The reductase reaction also conserves methionine for metabolic processes by preventing its loss as methionine sulfoxide. Methionine is an essential amino acid in mammals [13], required for protein initiation, incorporation into proteins, and one carbon metabolism. We hypothesized that the Msr contribute to normal nutrition, especially in animals living in the wild with limited food sources. We tested this hypothesis with a classical biochemical nutrition experiment, namely following the growth of weanling mice on diets with decreasing methionine content. We studied the growth of wild-type mice and of mice genetically modified to lack MsrA, MsrB1, or both and also of mice overexpressing MsrA. On a low methionine diet, we expected mice lacking the reductase to exhibit blunted growth and that this growth retardation might be prevented by overexpression of the reductase.

Materials and methods

Generation of Msr overexpressing and knockout mice

All mice described in this work were generated on C57BL/6 background. Mice were treated in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 85-23, 1996), and the study was approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute. MsrA transgenic mice were generated as described [14]. MsrA residues 21–233 were included in the construct TgCyto_Myr for the cytosolic targeted transgenic mice, and the overexpressed MsrA was

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[1] *Abbreviations used:* BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β synthase; GNMT, glycine *N*-methyltransferase; Msr, methionine sulfoxide reductase; KO_A, MsrA knockout; KO_B1, MsrB1 knockout; KO_AB1, MsrA and B1 double knockout; MS, methionine synthase; Ox, oxidizing species; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; TgCyto_Myr, transgenic methionine sulfoxide reductase targeted to the cytosol and myristoylated; Wt, wild type.

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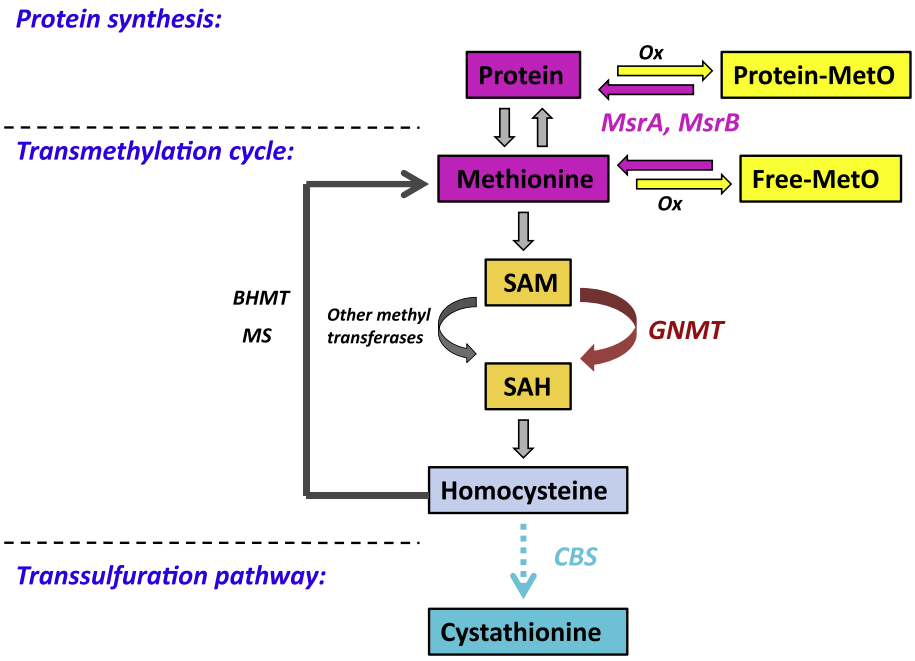

Fig. 1. Key components in the regulation of methionine metabolism. *In vivo*, both free and protein bound methionine can be oxidized by reactive oxygen and nitrogen species. MsrA and MsrB can reverse the methionine oxidation to replenish the methionine pool. The vast majority of methionine is found in proteins. The most important role of methionine in intermediary metabolism is in one carbon transfer. Methionine is required for generation of SAM, the major biological methyl donor *in vivo*. In the transmethylation cycle, SAM is converted to SAH, primarily by GNMT in the liver. SAH is further hydrolyzed to form homocysteine, which can be remethylated to regenerate methionine by MS or BHMT. Homocysteine can also undergo an irreversible transsulfuration pathway to form cystathionine. This also occurs mainly in the liver and is catalyzed by the enzyme CBS. The abbreviations are: MS, methionine synthase; BHMT, betaine-homocysteine methyltransferase; GNMT, glycine *N*-methyltransferase; CBS, cystathionine –synthase; Ox, oxidizing species; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

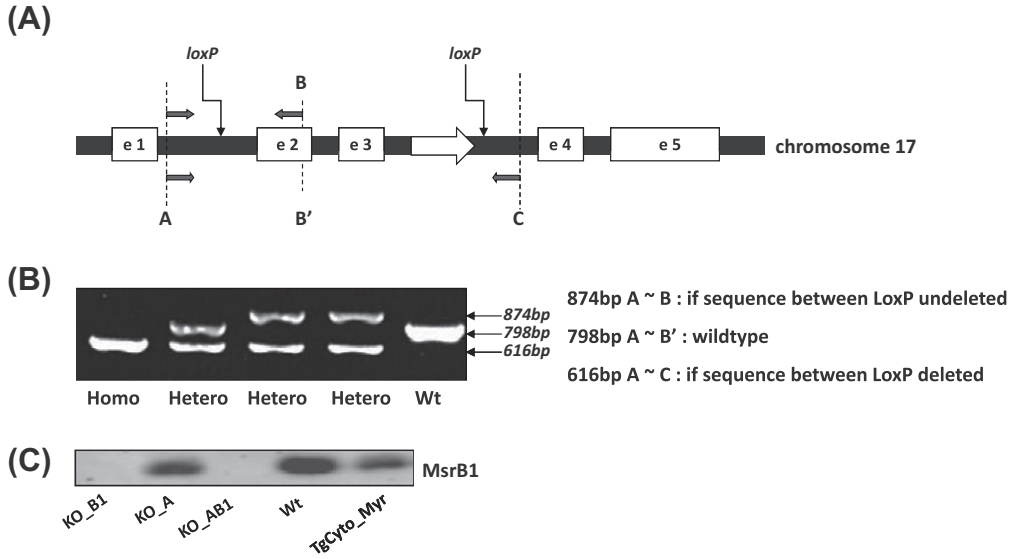

Fig. 2. Generation of MsrB1 and double knockout mice. (A) Scheme of the mouse MsrB1 gene and its flanking regions on chromosome 17. A segment with two LoxP sites flanking exon 2 and 3 replaced the endogenous sequence through homologous recombination (arrows). A, B, B′ and C mark the PCR primer annealing sites. (B) Tail PCR genotyping shows that both MsrB1 gene alleles were knocked out in the homozygous mouse (Homo) and one in the heterozygous (Hetero). (C) Immunoblot analysis of MsrB1 in liver tissues.

myristoylated *in vivo* as in the Wt [15]. Possible transgenic mice were screened for the presence of the transgene by PCR as described [14], and the founders were crossed with wild type C57BL/6 mice to generate transgenic lines. The KO_A mice used in this study were originally generated from a 129/SvJ background and later backcrossed for 10 generations into the C57BL/6 background [16]. Female mice heterozygous for the floxed exons 2 and 3 of the mice MsrB1 gene were generated by Ozgene (Bentley DC, WA, Australia) on a C57BL/6 background. Exon 2 and 3 of the MsrB1 gene were flanked by LoxP sites on chromosome 17 (Fig. 2A). Genomic excision of exon 2 and 3 of MsrB1 gene was accomplished by crossing with male B6.C-Tg (CMV-cre)1Cgn/J Cre recombinase expressing mice (006054, The Jackson laboratory, Bar Harbor, ME, USA). Offspring were genotyped by PCR using 3