Recognition of Age-damaged (R,S)-Adenosyl-\textit{L}-methionine by Two Methyltransferases in the Yeast \textit{Saccharomyces cerevisiae}\textsuperscript{*}

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The biological methyl donor \textit{S}-adenosylmethionine (AdoMet) can exist in two diastereoisomeric states with respect to its sulfonium ion. The \textit{S} configuration, (\textit{S},\textit{S})-AdoMet, is the only form that is produced enzymatically as well as the only form used in almost all biological methylation reactions. Under physiological conditions, however, the sulfonium ion can spontaneously racemize to the \textit{R} form, producing (\textit{R},\textit{S})-AdoMet. As of yet, (\textit{R},\textit{S})-AdoMet has no known physiological function and may inhibit cellular reactions. In this study, we found two \textit{Saccharomyces cerevisiae} enzymes that are capable of recognizing (\textit{R},\textit{S})-AdoMet and using it to methylate homocysteine to form methionine. These enzymes are the products of the \textit{SAM4} and \textit{MHT1} genes, identified previously as homocysteine methyltransferases dependent upon AdoMet and \textit{S}-methylmethionine, respectively. We found here that Sam4 recognizes both (\textit{S},\textit{S})- and (\textit{R},\textit{S})-AdoMet, but that its activity is much higher with the \textit{R},\textit{S} form. Mht1 reacts with only the \textit{R},\textit{S} form of AdoMet, whereas no activity is seen with the \textit{S},\textit{S} form. \textit{R},\textit{S}-Specific homocysteine methyltransferase activity is also shown here to occur in extracts of \textit{Arabidopsis thaliana}, \textit{Drosophila melanogaster}, and \textit{Caenorhabditis elegans}, but has not been detected in several tissue extracts of \textit{Mus musculus}. Such activity may function to prevent the accumulation of (\textit{R},\textit{S})-AdoMet in these organisms.

The aging process, as well as several human diseases, has been linked to the accumulation of spontaneously damaged biomolecules. Cells have evolved several ways of dealing with these altered molecules, including degradation, excretion, and repair pathways (1–6). The balance between the formation of age-altered molecules and the pathways that limit their cellular accumulation has been described as a battle between chemistry and biochemistry, where chemistry ultimately wins (2).

Although enzymes that recognize damaged DNA (3) and proteins (1, 2, 5) have been well characterized, this is not yet the case for spontaneously altered small molecules. Of the large number of metabolites that are produced and used by biological systems, many are unstable, degrading into forms that may have reduced function or that may be toxic. One pathway of small molecule degradation and cellular recognition has been described recently. Here, \textit{trans}-aconitate formed spontaneously from the citric acid cycle intermediate \textit{cis}-aconitate results in the inhibition of at least two steps in the cycle (7, 8). \textit{trans}-Aconitate is then recognized by a specific yeast methyltransferase; the methyl ester formed has reduced inhibitory properties (9).

One of the crucial small molecule metabolites in all organisms is \textit{S}-adenosyl-\textit{L}-methionine (AdoMet)\textsuperscript{2} (10–12). Second to ATP, it is probably the most widely used cofactor in nature (12, 13). Not only does it serve as the primary methyl donor, but it also functions as an amino, adenosyl, and ribosyl donor (11). It also plays a role in the formation of adenosyl radicals (14) and as a precursor of polyamines (15). AdoMet has been shown to be unstable in cells, forming a variety of degradation products. Internal cyclization can form homoserine lactone and \textit{S}-methylothioadenosine; hydrolysis at the glycosidic bond can form adenine and \textit{S}-pentosylmethionine; and racemization at the sulfonium ion can form the \textit{R},\textit{S} diastereomer (16–18). We have been particularly interested in this latter reaction because pathways for the utilization or metabolism of this diastereomer have not been described.

As biosynthesized, AdoMet is in the \textit{S},\textit{S} configuration, where the first \textit{S} corresponds to the stereochemistry at the sulfonium ion and the second to that at the \textit{\alpha}-carbon (19, 20). The \textit{S},\textit{S} form appears to be the biologically active species (21, 22), and a number of methyltransferases have been shown to use it exclusively (20, 21, 23, 24). However, the instability of the sulfonium center results in spontaneous racemization under biological conditions to form (\textit{R},\textit{S})-AdoMet (Fig. 1) (20). If only the \textit{S},\textit{S} form is used, and the \textit{R},\textit{S} form is constantly produced by racemization, the levels of biologically inactive (\textit{R},\textit{S})-AdoMet should build up over time in cells. This material may not only take up precious cell space, but may also be toxic (24, 25). The racemization of AdoMet may also be a factor in its pharmacology when used as a nutritional supplement (SAMe) (26). These preparations generally contain from 20 to 40% of the \textit{R},\textit{S} form (27), and there is uncertainty at present about the relative contribution of each form to the therapeutic effect and whether there may be any toxicity associated with the \textit{R},\textit{S} form.

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\textsuperscript{2}The abbreviations used are: AdoMet, \textit{S}-adenosyl-\textit{L}-methionine; HPLC, high performance liquid chromatography; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol; AdoHcy, \textit{S}-adenosyl-\textit{L}-homocysteine.
There is some indirect evidence to suggest that (R,S)-
AdoMet can be metabolized in cells. Based on steady-state calculations for cells at physiological temperature and pH, the ratio of (R,S) to (S,S)-AdoMet has been calculated as 19:81 (18). However, the R,S form is undetectable in soybean extracts (17), and the R,S/S,S ratio in mouse liver (18) and rat brain (25) has been measured as 3:97. Thus, there appears to be a mechanism(s) that keeps the intracellular (R,S)-AdoMet levels low.

We were thus interested in an older report that suggested that (R,S)-AdoMet might be utilized by a yeast methyltransferase (23). In this work, homocysteine methyltransferase activity was observed at twice the rate when an S,S/R,S mixture of AdoMet was used compared with that seen with the S,S form at an equal total concentration (23). Previous studies have shown that there are at least two homocysteine methyltransferase enzymes present in Saccharomyces cerevisiae (28, 29). These are encoded by the SAM4 and MHT1 genes and utilize AdoMet and S-methylmethionine, respectively, as methyl donors (29). It is not clear whether either of these enzymes was responsible for the utilization of (R,S)-AdoMet seen initially (23).

We were intrigued by the apparent lack of cellular logic in encoding an AdoMet-utilizing homocysteine methyltransferase. Homocysteine methyltransferases function to make methionine, which is then the precursor of AdoMet. The methionine used to make AdoMet would be regenerated by the methyltransferase activity, and the homocysteine (Hcy) used by the methyltransferase (MT) would be regenerated by the hydrolase. The combination of activities of these three enzymes would be expected to result in the net hydrolysis of ATP to form adenosine. Adenosine requires three ATP molecules to be regenerated as ATP.

**EXPERIMENTAL PROCEDURES**

**Preparation and Purification of (R,S)- and (S,S)-[^3]H]AdoMet—
S-Adenosyl-L-[methyl-^3]H]methionine (referred to throughout as [^3]H]AdoMet; 1 mCi/ml, 79.0 Ci/mmol, in dilute HCl/ethanol (9:1, v/v) at pH 2–2.5; GE Healthcare) was diluted 10-fold in 0.1 M HCl and divided into two aliquots. One aliquot was incubated for 8 days at 37 °C, resulting in an R,S-enriched [^3]H]AdoMet sample, whereas the other was kept at −80 °C. An aliquot (100 μl) of each preparation was then fractionated with a cation-exchange HPLC column (Whatman Partisil SCX, 10-μm bead diameter, 4.6 mm, inner diameter, × 250 mm) using a method similar to that described previously (18). Briefly, the column was equilibrated and eluted with 80% buffer A (0.7 ml of concentrated NH₄OH added to 1000 ml of 20% acetonitrile in H₂O and brought to pH 3.0 with 88% acetic acid) and 20% buffer B (buffer A adjusted to 50 mM (NH₄)₂SO₄ and then to pH 3.0 with concentrated sulfuric acid). The column was eluted at room temperature at a flow rate of 1 ml/min. The absorbance of the eluate was monitored at 254 nm, and 500-μl fractions were collected. An aliquot (50 μl) of each fraction was mixed with 2 ml of fluor (Safety-Solve, Research Products International Corp., Mount Prospect, IL) to measure radioactivity. The fractions corresponding to the R,S peak of the incubated sample and to the S,S peak of the non-incubated sample were each

![FIGURE 1. S,S and R,S diastereomers of AdoMet. In the S,S/R,S notation, the first letter designates the stereoconfiguration of the sulfonium ion, and the second letter designates that of the α-carbon of the methionine moiety. (S,S)-AdoMet is the product of the AdoMet synthetase reaction and is the form predominantly used in enzymatic reactions. (R,S)-AdoMet forms spontaneously under physiological conditions from (S,S)-AdoMet.](image1)

![FIGURE 2. AdoMet-dependent homocysteine methyltransferase, in combination with AdoMet synthetase and AdoHcy hydrolase, catalyzes a potential futile cycle. The methionine used to make AdoMet would be regenerated by the methyltransferase activity, and the homocysteine (Hcy) used by the methyltransferase (MT) would be regenerated by the hydrolase. The combination of activities of these three enzymes would be expected to result in the net hydrolysis of ATP to form adenosine. Adenosine requires three ATP molecules to be regenerated as ATP.](image2)
TABLE 1

| Yeast strains | Genotype | Source |
|---------------|----------|--------|
| BY4741        | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | SGD² |
| mht1¹ (BY4741) | BY4741, Δyl062c::Kan² | SGD² |
| BY4742        | MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 | SGD² |
| sam4 (BY4742) | BY4742, Δylp173c::Kan² | SGD² |
| mht1 (BY4742) | BY4742, Δylp062c::Kan² | SGD² |
| sam4⁻ mht1⁻ | MATa Δylp273c::Kan² Δylp062c::Kan² | LYS² MET² |

¹ Strains prepared by the Saccharomyces Genome Deletion Project (SGDP) and purchased from Invitrogen.

Results

Recognition of Age-damaged (R,S)-AdoMet

Yeast strains

Table 1 lists the yeast strains used in this study. The sam4⁻ and mht1⁻ single knock-out mutant strains were generated by the Saccharomyces Genome Deletion Project (www-sequence.stanford.edu/group/yeast_deletion_project/available.html) and purchased from Invitrogen. The sam4⁻/mht1⁻ double knock-out strain was created by mating the MATa strain of sam4⁻ with the MATa strain of mht1⁻ and selecting for cells able to grow on lysine- and methionine-deficient plates. The resulting diploid strain was induced to sporulate, and the haploid spores containing both gene deletions were identified by screening on kanamycin plates for rough and smooth phenotypes. The resulting diploid strain was induced to sporulate, and the haploid spores containing both gene deletions were identified by screening on kanamycin plates for rough and smooth phenotypes.

Preparation of (R,S)- and (S,S)-³H]AdoMet—In preparing (R,S)-AdoMet, we took advantage of the fact that (S,S)-AdoMet can spontaneously racemize to the RS form in vitro under physiological conditions to produce an SS/RS mixture that is roughly 50:50 (16–18). In Fig. 3, we compare the ³H NMR spectra of a commercial preparation of AdoMet with and without incubation to induce racemization, which was performed at pH 10 to minimize the formation of other AdoMet degradation products such as methythioadenosine and homoserine lactone (18). Clear peaks associated with the methyl group of AdoMet were found at 2.93 ppm in (S,S)-AdoMet and at 2.89 ppm in (R,S)-AdoMet (27). Under our conditions, we obtained a 49:51 mixture that was close to the equilibrium distribution. We then used these conditions to generate (R,S)-³H]AdoMet from a preparation of (S,S)-³H]AdoMet (Fig. 4). We were able to cleanly separate the isotonically labeled RS and SS forms by
Non-isotopically labelled commercial AdoMet (no treatment)

![HPLC chromatogram](image)

**FIGURE 3.** 400 MHz 1H NMR spectra of commercial and racemized AdoMet. A, AdoMet was analyzed directly. The peaks at 2.93 and 2.89 ppm correspond to the hydrogens of the sulfonium-bound methyl groups of (S,S)- and (R,S)-AdoMet, respectively. B, AdoMet was analyzed after incubation in 0.1 M HCl for 160 h at 37 °C.

B. Incubated 7 days at 37°C and pH 1.0

![HPLC chromatogram](image)

A. Non-isotopically labelled commercial AdoMet (no treatment)

![HPLC chromatogram](image)

**FIGURE 4.** Purification of (S,S)- and (R,S)-[3H]AdoMet. (S,S)-[3H]AdoMet and R,S-enriched [3H]AdoMet were fractionated by cation-exchange chromatography using a Partisil SCX HPLC column (4.6 mm, inner diameter, ×250 mm). R,S-Enriched [3H]AdoMet was prepared by incubation at 37 °C for 8 days at pH 1.0. Fractions (1 ml) were collected, and 50 μl was counted in 2 ml of scintillation fluid. [ ] radioactivity from the S,S sample; [ ] radioactivity from the R,S-enriched sample. Fractions corresponding to the S,S and R,S peaks were pooled and stored at -80°C.

A. Non-isotopically labelled commercial AdoMet (no treatment)

![HPLC chromatogram](image)

**FIGURE 5.** Confirmation of the configuration of purified (S,S)- and (R,S)-[3H]AdoMet by their utilization by the protein l-isoaaspartyl O-methyltransferase. Human recombinant l-isoaaspartyl protein methyltransferase activity was measured in the presence and absence of the peptide substrate KASA(isoD)LAKY using 0.016 pmol of either (S,S)- or (R,S)-[3H]AdoMet prepared as described in the legend to Fig. 4. Assays were done in triplicate, and error bars represent S.D.

**SUBSTRATES**

![Graph](image)

**FIGURE 5.** Confirmation of the configuration of purified (S,S)- and (R,S)-[3H]AdoMet by their utilization by the protein l-isoaaspartyl O-methyltransferase. Human recombinant l-isoaaspartyl protein methyltransferase activity was measured in the presence and absence of the peptide substrate KASA(isoD)LAKY using 0.016 pmol of either (S,S)- or (R,S)-[3H]AdoMet prepared as described in the legend to Fig. 4. Assays were done in triplicate, and error bars represent S.D.

Recognition of Age-damaged (R,S)-AdoMet

To confirm the identity of the labeled (R,S)- and (S,S)-AdoMet samples obtained from the HPLC purification, we tested their ability to serve as substrates for a typical AdoMet-dependent methyltransferase that would be expected to use the S,S form. Fig. 5 shows that the human recombinant l-isoaaspartyl protein repair methyltransferase uses purified (S,S)-[3H]AdoMet essentially to completion (94%), whereas no use is made of purified (R,S)-[3H]AdoMet.

Yeast Sam4 and Mht1 Catalyze R,S-Dependent Homocysteine Methyltransferase Activity—We first investigated whether the extracts of wild-type *S. cerevisiae* could use (R,S)-AdoMet as a methyl donor in the conversion of homocysteine to methionine. We found that this was the case; after 60 min of incubation, we could quantitatively convert (R,S)-[3H]AdoMet to a neutral species consistent with [3H]methionine (Fig. 6). We found a similar result for (S,S)-[3H]AdoMet, indicating that both diastereomers could be utilized as methyl donors for yeast homocysteine methyltransferases. Control reactions in the absence of homocysteine revealed little or no methylation activity (Fig. 6), confirming the specificity of the assay. We also confirmed that the product was [3H]methionine in each case by co-chromatography of the product with a methionine standard by thin-layer chromatography using silica plates and a solvent system of n-butyl alcohol/acetic acid/water (4:1:1) (data not shown).

Two homocysteine methyltransferases have been described in yeast, the products of the *SAM4* and *MHT1* genes (29). Thus, we measured the contribution of each of these enzymes to (R,S)-AdoMet utilization. When extracts of a *sam4* knockout strain were incubated with (S,S)-[3H]AdoMet, we found no homocysteine-dependent methyltransferase activity (Fig. 6), suggesting that (S,S)-AdoMet utilization in homocysteine methylation in yeast is limited to the Sam4 enzyme. On the other hand, homocysteine-dependent methyltransferase activity with (R,S)-[3H]AdoMet, although decreased, was still present (Fig. 6). This result provides the first direct evidence that the “degraded” R,S form of AdoMet may be utilized in a specific methyltransferase reaction. This result also suggests that, although Sam4 is responsible for most of the (R,S)-AdoMet-dependent homocysteine methyltransferase activity, there is at least one additional activity present that can utilize the R,S diastereomer, but not the S,S diastereomer, of AdoMet for methionine synthesis.
We thus proceeded to determine whether Mht1 is responsible for all or part of the Sam4-independent homocysteine methyltransferase activity. We first analyzed activity in an extract of an mht1- deletion strain. We detected little change in activity with either (R,S)- or (S,S)-[3H]AdoMet, confirming the major role of the Sam4 enzyme. We then prepared a sam4- / mht1- double knock-out strain. Extracts of this strain were found to be incapable of catalyzing homocysteine methylation with either (R,S)- or (S,S)-[3H]AdoMet (Fig. 6). This experiment demonstrates that the utilization of (R,S)-AdoMet for methionine synthesis in the sam4- extract is due to the Mht1 protein. This protein was demonstrated previously to catalyze homocysteine methylation using S-methylmethionine as a methyl donor (29). These results show that it can also utilize (R,S)-AdoMet. Overall, our data suggest that Sam4 and Mht1 are responsible for the (R,S)-AdoMet-dependent homocysteine methyltransferase activity seen in S. cerevisiae and that Sam4 is responsible for the activity with (S,S)-AdoMet.

Because zinc has been shown to play a role in the homocysteine-binding site of other methionine-forming enzymes (38, 39), we investigated whether the activities of the yeast enzymes might be enhanced by the addition of zinc ion to the assay mixture. However, in experiments with mht1- and sam4- extracts, we found no effect of the addition of either 1 mM zinc chloride or 1 mM sodium EDTA on the homocysteine methyltransferase activities (data not shown).

Kinetics of Mht1 and Sam4 AdoMet-dependent Homocysteine Methyltransferases—To better understand the importance of these enzymes in vivo, we characterized the kinetics of the Mht1 and Sam4 methyltransferases in vitro. Using sam4- extracts as a source of the Mht1 enzyme, we confirmed that this enzyme is specific for the R,S form of AdoMet (Fig. 7A). Similarly, using mht1- extracts, we demonstrated the ability of Sam4 to use both forms of AdoMet (Fig. 7B). The maximal velocities of these enzymes and their $K_m$ values for homocysteine from these data are compared in Table 2. In these experiments, the presence of other components in the cell extracts may have influenced the observed $K_m$ and $V_{max}$ values, but would be expected to reflect the situation in intact cells. We found that, although Sam4 is capable of using both (S,S)- and (R,S)-AdoMet as substrates, it has a 2.5-fold higher activity with the $R,S$ form (Table 2). The $K_m$ for homocysteine is similar with both metal donors, ranging from 32 to 44 μM, resulting in a catalytic efficiency ($V_{max}/K_m$) with (R,S)-AdoMet that is 3.3-fold larger than that with (S,S)-AdoMet. These results suggest that the predominant physiological substrate of the Sam4 homocysteine methyltransferase is the racemized (R,S)-AdoMet form.

Using (R,S)-AdoMet as the methyl donor, we found that the Mht1 enzyme has a $K_m$ for homocysteine of 45 μM, a value similar to those of the Sam4 enzyme with (R,S)- and (S,S)-AdoMet (Table 2). However, we found that the catalytic efficiency of Mht1 with the $R,S$ form is 27-fold less than that of
Sam4 for the R,S form. Mht1 has little or no detectable activity for the S,S form.

Homocysteine Methyltransferase Activities That Utilize the R,S Form of AdoMet Are Present in a Variety of Organisms—Genes that are homologous to the yeast SAM4 and MHT1 genes are present in Arabidopsis, Drosophila, and Escherichia coli (29, 40). Using a series of BLAST searches, we determined that there are at least three homologous genes present in Arabidopsis, two in Drosophila, two in C. elegans, and three in mice (Fig. 8). We thus looked for R,S- and S,S-dependent AdoMet activity in extracts of these organisms. In embryonic Drosophila extract, a situation similar to that in yeast was observed in which both the S,S and R,S forms of AdoMet were used by the extract to methylate homocysteine, with a similar preference for the R,S form (Fig. 9) (cf. Fig. 6). Unlike the situation seen in yeast or Drosophila, Arabidopsis seed extract had little or no detectable homocysteine methyltransferase activity with (S,S)-AdoMet (Fig. 9). However, (R,S)-AdoMet was found to be an effective substrate (Fig. 9). Similar R,S-specific activity could be seen with the C. elegans extract.

**TABLE 2**

Kinetic values of the Mht1 and Sam4 homocysteine methyltransferases with (R,S)- or (S,S)-AdoMet in cell extracts

| Enzyme  | (R,S)-AdoMet | (S,S)-AdoMet |
|---------|--------------|--------------|
|         | $K_m$ for Hcy | $V_{max}$   | $V_{max}/K_m$ | $K_m$ for Hcy | $V_{max}$   | $V_{max}/K_m$ |
| Mht1    | 0.045 ± 0.015 | 0.0065 ± 0.008 | 0.015 | 0.044 ± 0.007 | 0.052 ± 0.003 | 1.2 |
| Sam4    | 0.032 ± 0.009 | 0.13 ± 0.02 | 4.0 |

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Unexpectedly, no AdoMet-dependent homocysteine methyltransferase activity was observed in mouse brain extract (Fig. 9) or in extracts of liver, kidney, and heart tissue (data not shown). The only gene products in mouse with apparent homology to the two yeast gene products are the N\textsuperscript{5}-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase), the betaine-specific homocysteine methyltransferase (BHMT1), and the BHMT2 product (Fig. 8). These latter enzymes appear to be more evolutionarily distant and appear to have lost the ability to utilize (R,S)-AdoMet as a methyl donor. We performed control experiments to show that both methionine synthase and BHMT2 were active in our preparation of mouse liver extracts. Methionine synthase was assayed as described (41); BHMT2 was assayed using S-methylmethionine as a substrate (42) in the presence of the BHMT1-specific homocysteine methyltransferase inhibitor S-(\textit{\textbeta}-carboxybutyl)-1-homocysteine (43, 44).3 Because the conserved C-terminal cysteine residues of the enzyme homologs (Fig. 8) are associated with bound zinc ions in the active site in some of these enzymes (38–40), we performed controls in which reaction mixtures of (R,S)-AdoMet and mouse liver extracts were supplemented with 1 mM zinc. However, no activity was observed (data not shown). If there are enzymes in mice that can use (R,S)-AdoMet or (S,S)-AdoMet to methylate homocysteine, we could not detect their activity under our conditions.

**DISCUSSION**

In this work, we have provided evidence that at least two enzymes in yeast are capable of recognizing the age-damaged R,S form of AdoMet. These enzymes catalyze the formation of methionine from homocysteine utilizing the methyl group of (R,S)-AdoMet in a reaction that converts it to S-adenosyl-1-homocysteine (AdoHcy) for further metabolism to adenosine and homocysteine. The combined activities of the Mht1 and Sam4 enzymes, along with AdoMet synthetase and AdoHcy hydrolase, would be expected to catalyze the ATP-dependent conversion of (R,S)-AdoMet to (S,S)-AdoMet (Figs. 2 and 10). It is not clear at this point how effective these reactions are at preventing the accumulation of damaged AdoMet and whether these methyltransferases are the only activities in cells that can recognize (R,S)-AdoMet (Fig. 10).

In \textit{E. coli}, the HemN coproporphyrin III oxidase has been crystallized with either (R,S)- or (S,S)-AdoMet in the active site adjacent to a [4Fe-4S] cluster (45). It has been speculated that this enzyme may be able to interconvert the R,S and S,S forms (45), although it is unclear what type of mechanism may drive

\[ \text{(S,S)AdoMet (Active Cofactor)} \]

\[ \xrightarrow{\text{Spontaneous}} \]

\[ \text{(R,S)AdoMet (Inactive Cofactor)} \]

\[ \xrightarrow{\text{Hcy}} \]

\[ \text{Sam4/Mht1} \]

\[ \text{Met} \]

\[ \text{AdoHcy} \]

\[ ? \]

3 T. A. Garrow, personal communication.
the formation of (S,S)-AdoMet and whether such an activity is present in other organisms.

We have shown here that (R,S)-AdoMet-specific homocysteine methyltransferase activities exist in a variety of species in addition to yeast, including plants and invertebrates. However, we found no evidence for such activities in several mouse tissues. It is thus unclear how mammalian cells metabolize (R,S)-AdoMet. In bacterial cells, the YagD protein can utilize not only S-methylmethionine as a methyl donor to either homocysteine or selenocysteine, but also a fraction of an AdoMet preparation that has been suggested to represent the R,S form (28, 29). Similar results have been found with a selenocysteine methyltransferase from the legume Astragalus bisulcatus that is homologous to the yagD gene of E. coli, suggesting that both enzymes may preferentially use (R,S)-AdoMet (28). It is interesting to note that S-methylmethionine-specific homocysteine methyltransferases from Arabidopsis transfer the pro-R-methyl group from S-methylmethionine that corresponds to the sulfonium-bound methyl group from (R,S)-AdoMet (40, 46).

Biochemical and sequence studies have shown that homologous homocysteine methyltransferases in nature can utilize a variety of methyl donors (Table 3). These enzymes do not have sequences similar to those of the seven-stranded β-sheet methyltransferase enzymes or other known types of methyltransferases (50) and appear to represent a new class of methyltransferases based on binding the methyl-accepting substrate and not the specific methyl-donating substrate. Although it has been reported that the three Arabidopsis enzymes use (S,S)-AdoMet (40, 47), the fact that we observed R,S-specific, and not S,S-specific, AdoMet-dependent homocysteine methyltransferase activity in Arabidopsis extracts suggests that these three enzymes might actually be more specific for (R,S)-AdoMet. It is also of interest that the closest mammalian homologs of the yeast Sam4 and Mht1 enzymes in mammals use N⁵-methyltetrahydrofolate (48), betaine (49), or S-methylmethionine (42) as a methyl-donating substrate (Table 3). The fact that (R,S)-AdoMet-dependent methyltransferase activity was observed in yeast, Drosophila, nematodes, and plants but not in mammals is puzzling, but suggests that the mammalian enzymes may have enhanced specificity for the methyl donor. Interestingly, it has been reported that the human BHMT2 enzyme has a trace activity with AdoMet (42); further investigation is warranted here.

The utilization by the yeast Sam4 enzyme of both (S,S)- and (R,S)-AdoMet is of interest because Sam4 expression levels increase when yeast is grown in the presence of high levels of methionine (29). Because most enzymes involved in methionine synthesis, including Mht1, are down-regulated under this condition, Sam4 may have an additional role of regulating AdoMet when it is present in excess (29). If this is indeed the case, it would be an added benefit for this enzyme to deplete cells of both the R,S and S,S forms.

Although much is known about the biological pathways in which the S,S form of AdoMet participates, the cellular interactions and metabolism of its R,S degradation product are only beginning to be understood. We still need to know how “toxic” (R,S)-AdoMet is. It has been reported that (R,S)-AdoMet inhibits the (S,S)-AdoMet-dependent activities of catechol O-methyltransferase, histamine N-methyltransferase, hydroxyindole O-methyltransferase, and phenylethanolamine N-methyltransferase (24). However, another study demonstrated no effect of (R,S)-AdoMet on the activity of the latter enzyme (25). (R,S)-AdoMet may also be responsible for the generation of other types of cellular inhibitors. For example, the enzyme 1-aminocyclopentane-1-carboxylate synthase usually reacts with (R,S)-AdoMet to produce 1-aminocyclopentane-1-carboxylate and 5'-methylthioadenosine. However, with (R,S)-AdoMet, it can form 1-vinylglycinine as a suicide substrate inhibitor (51, 52). Although we are only in the initial stages of understanding how (R,S)-AdoMet may affect cellular function, the results presented in this study suggest that a variety of cell types contain enzymes that can specifically recognize it and convert it to molecules that can be readily metabolized. As such, these (R,S)-AdoMet-dependent homocysteine methyltransferases can be seen as a line of defense against the spontaneous accumulation of a potentially harmful molecule.

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REFERENCES

1. Martindale, J. L., and Holbrook, N. J. (2002) J. Cell. Physiol. 192, 1–15
2. Clarke, S. (2003) Ageing Res. Rev. 2, 263–285
3. Lombard, D. B., Chua, K. F., Mostoslavsky, R., Franco, S., Gostissa, M., and Alt, F. W. (2005) Cell 120, 497–512
4. Frietet, B. (2006) FEBS Lett. 580, 2910–2916
5. Veiga da Cunha, M., Jacquotin, P., Delpeyrre, G., Godfraind, C., Theate, I., Vertommen, D., Clotman, F., Lemaigre, F., Devuyst, O., and Van Schaftingen, E. (2006) Biochem. J. 399, 257–264
6. Hernebring, M., Brogren, G., Aguían, H., Semb, H., and Nyström, T. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 7700–7705
7. Lauble, H., Kennedy, M. C., Beinert, H., and Stout, C. D. (1994) J. Mol. Biol. 237, 437–451
8. Rebholz, K. L., and Northrop, D. B. (1994) Arch. Biochem. Biophys. 312, 227–233
9. Cai, H., Strouse, J., Dumlao, D., Jung, M. E., and Clarke, S. (2001) Biochemistry 40, 2210–2219
10. Loenen, W. A. (2006) Biochem. Soc. Trans. 34, 330–333
11. Fontecave, M., Atta, M., and Mulliez, E. (2004) Trends Biochem. Sci. 29, 243–249
12. Lu, S. C. (2000) Int. J. Biochem. Cell Biol. 32, 391–395
13. Cantoni, G. L. (1975) Annu. Rev. Biochem. 44, 435–451
14. Jarrett, J. T. (2003) Curr. Opin. Chem. Biol. 7, 174–182
15. Urdiales, J. I., Medina, M. A., and Sanchez-Jimenez, F. (2001) Eur. J. Gastroenterol. Hepatol. 13, 1015–1019
16. Wu, S. E., Huskey, W. P., Borchardt, R. T., and Schwenk, R. L. (1983) Biochemistry 22, 2828–2832
17. Creason, G. L., Madison, I. T., and Thompson, J. F. (1985) Phytochemistry 24, 1115–1155
18. Hoffman, J. L. (1986) Biochemistry 25, 4444–4449
19. Cornforth, J. W., Reichard, S. A., Talayad, P., Carrell, H. L., and Glusker, J. P. (1977) J. Am. Chem. Soc. 99, 7200–7300
20. De la Haba, G., Jamieson, G. A., Mudd, S. H., and Richards, H. H. (1959) J. Am. Chem. Soc. 81, 3975–3980
21. Cannon, L. M., Butler, F. N., Wan, W., and Zhou, Z. S. (2002) Anal. Biochem. 308, 358–363
22. Bentley, R. (2005) Chem. Soc. Rev. 34, 609–624
23. Zappia, V., Zydek-Cwic, C. R., and Schlenk, F. (1969) Biochim. Biophys. Acta 178, 185–187
24. Borchardt, R. T., and Wu, Y. S. (1976) J. Med. Chem. 19, 1099–1103
25. Beaudouin, C., Haurat, G., Laffitte, J. A., and Renaud, B. (1993) J. Neurochem. 61, 928–935
26. Bottiglieri, T. (2002) Am. J. Clin. Nutr. 76, 1151S–1157S
27. Hanna, G. M. (2004) Pharmazie 59, 251–256
28. Neuhierl, B., Thanbichler, M., Lottspeich, F., and Bock, A. (1999) J. Biol. Chem. 274, 5407–5414
29. Thomas, D., Becker, A., and Sirdin-Kerjan, Y. (2000) J. Biol. Chem. 275, 40718–40724
30. Lowenson, J. D., and Clarke, S. (1991) J. Biol. Chem. 266, 19396–19406
31. Porras-Yakushi, T. R., Whitegegge, J. P., Miranda, T. B., and Clarke, S. (2005) J. Biol. Chem. 280, 34590–34598
32. Lowery, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
33. Villa, S. T., Xu, Q., Downie, A. B., and Clarke, S. G. (2006) Physiol. Plant. 128, 581–592
34. Miranda, T. B., Lowenson, J. D., and Clarke, S. (2004) FEBS Lett. 577, 181–186
35. Kagan, R. M., and Clarke, S. (1995) Biochemistry 34, 10794–10806
36. Fellner, T., Piribauer, P., and Ogris, E. (2003) Methods Enzymol. 366, 187–203
37. Shapiro, S. K., Yphantis, D. A., and Almenas, A. (1964) J. Biol. Chem. 239, 1551–1556
38. Peariso, K., Gouding, C. W., Huang, S., Matthews, R. W., and Penner-Hahn, J. E. (1998) J. Am. Chem. Soc. 120, 8410–8416
39. Evans, J. C., Huddler, D. P., Jiracek, J., Castro, C., Millian, N. S., Garrow, T. A., and Ludwig, M. L. (2002) Structure (Camb.) 10, 1159–1171
40. Ranocha, P., Bourgis, F., Ziemak, M. J., Rhodes, D., Gage, D. A., and Hanson, A. D. (2000) J. Biol. Chem. 275, 15962–15968
41. Banerjee, R., Chen, Z., and Gullati, S. (1997) Methods Enzymol. 281, 189–196
42. Garrow, T. A., Szegedi, S. S., and Castro, C. (2006) FASEB J. 20, A606 (abstr.)
43. Castro, C., Gratson, A. A., Evans, J. C., Jiracek, J., Collinson, M., Ludwig, M. L., and Garrow, T. A. (2004) Biochemistry 43, 5341–5351
44. Jiracek, J., Collinson, M., Rosenberg, I., Budesinsky, M., Protivinska, E., Netusilova, H., and Garrow, T. A. (2006) J. Med. Chem. 49, 3982–3989
45. Layer, G., Moser, J., Heinz, D. W., Jahn, D., and Schubert, W. D. (2003) EMBO J. 22, 6214–6224
46. Grue-Sorensen, G., Kelstrup, E., Kjaer, A., and Madsen, J. O. (1984) J. Chem. Soc. Perkin Trans. 1, 1091–1097
47. Ranocha, P., McNeil, S. D., Ziemak, M. J., Li, C., Tarczynski, M. C., and Hanson, A. D. (2001) Plant J. 25, 575–584
48. Loughlin, R. E., Elford, H. L., and Buchanan, J. M. (1964) J. Biol. Chem. 239, 2888–2895
49. Garrow, T. A. (1996) J. Biol. Chem. 271, 22831–22838
50. Schubert, H. L., Blumenthal, R. M., and Cheng, X. (2003) Trends Biochem. Sci. 28, 329–335
51. McCarthy, D. L., Capitani, G., Feng, L., Gruetter, M. G., and Kirsch, J. F. (2001) Biochemistry 40, 12276–12284
52. Satoh, S., and Yang, S. F. (1989) Plant Physiol. 91, 1036–1039