Recognition of Age-damaged (R,S)-Adenosyl-L-methionine by Two Methyltransferases in the Yeast Saccharomyces cerevisiae

Chris R. Vinci and Steven G. Clarke

From the Department of Chemistry and Biochemistry and the Molecular Biology Institute, UCLA, Los Angeles, California 90095-1569

The biological methyl donor S-adenosylmethionine (AdoMet) can exist in two diastereoisomeric states with respect to its sulfonium ion. The S configuration, (S,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 R form, producing (R,S)-AdoMet. As of yet, (R,S)-AdoMet has no known physiological function and may inhibit cellular reactions. In this study, we found two Saccharomyces cerevisiae enzymes that are capable of recognizing (R,S)-AdoMet and using it to methylate homocysteine to form methionine. These enzymes are the products of the SAM4 and MHTT1 genes, identified previously as homocysteine methyltransferases dependent upon AdoMet and S-methylmethionine, respectively. We found here that Sam4 recognizes both (S,S)- and (R,S)-AdoMet, but that its activity is much higher with the R,S form. Mht1 reacts with only the R,S form of AdoMet, whereas no activity is seen with the S,S form. R,S-Specific homocysteine methyltransferase activity is also shown here to occur in extracts of Arabidopsis thaliana, Drosophila melanogaster, and Caenorhabditis elegans, but has not been detected in several tissue extracts of Mus musculus. Such activity may function to prevent the accumulation of (R,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, trans-aconitate formed spontaneously from the citric acid cycle intermediate cis-aconitate results in the inhibition of at least two steps in the cycle (7, 8). 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 S-adenosyl-L-methionine (AdoMet) (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 5′-methylthioadenosine; hydrolysis at the glycosidic bond can form adenine and S-pentosylmethionine; and racemization at the sulfonium ion can form the R,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 S,S configuration, where the first S corresponds to the stereochemistry at the sulfonium ion and the second to that at the α-carbon (19, 20). The S,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 (R,S)-AdoMet (Fig. 1) (20). If only the S,S form is used, and the R,S form is constantly produced by racemization, the levels of biologically inactive (R,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 R,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 R,S form.

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1 To whom correspondence should be addressed: Dept. of Chemistry and Biochemistry and the Molecular Biology Inst., UCLA, 607 Charles E. Young Dr. East, Los Angeles, CA 90095-1569. Tel.: 310-825-8754; Fax: 310-825-1968; E-mail: clarke@mbi.ucla.edu.

2 The abbreviations used are: AdoMet, S-adenosyl-L-methionine; HPLC, high performance liquid chromatography; BisTris, 2-(bis[2-hydroxyethyl]amino)-2-(hydroxymethyl)propane-1,3-diol; AdoHcy, S-adenosyl-L-homocysteine.
There is some indirect evidence to suggest that (R,S)-
AdoMet can be metabolized in cells. Based on steady-state cal-
culations 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 mecha-
nism(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 methyltrans-
ferase (23). In this work, homocysteine methyltransferase activ-
ity 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 methyltrans-
ferase. Homocysteine methyltransferases function to make
methionine, which is then the precursor of AdoMet. The source
of the methyl group can be $N^5$-methyltetrahydrofolate, $S$-
methylmethionine, or betaine (12, 29). If AdoMet itself
was used as the donor, the reaction would appear to be a futile
cycle, resulting in the hydrolysis of 3 ATP eq (Fig. 2). However,
such a “futility cycle” could be justified if the AdoMet-dependent
homocysteine methyltransferase utilized the R,S form rather
than the S,S form. In this scenario, the inactive R,S form, result-
ing from the unwanted racemization of the S,S form, would be
converted to methionine, which could in turn be converted to
the active S,S form of AdoMet. Such a mechanism could explain
the low cellular levels of (R,S)-AdoMet observed (17, 18, 25).
In this work, we demonstrate that Sam4 and Mht1 in S. cer-
eviseae are both in fact capable of using (R,S)-AdoMet as a
methyl donor. Sam4 has a higher specificity for (R,S)-
than for (S,S)-AdoMet, and Mht1 uses (R,S)-AdoMet in exclusion of the
S,S form. Thus, these two enzymes may work to prevent the
accumulation of (R,S)-AdoMet within cells.
TABLE 1  
Yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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<tbody>
<tr>
<td>BY4741</td>
<td>MATa his3Δ1 leu2Δ1 met15Δ0 ura3Δ0</td>
<td>SGDp*</td>
</tr>
<tr>
<td>mht1Δ (BY4741)</td>
<td>BY4741, Δyl062c::Kan'</td>
<td>SGDp*</td>
</tr>
<tr>
<td>BY4742</td>
<td>MATa his3Δ1 leu2Δ1 lys2Δ0 ura3Δ0</td>
<td>SGDp*</td>
</tr>
<tr>
<td>sam4Δ (BY4742)</td>
<td>BY4742, Δylp273w::Kan'</td>
<td>SGDp*</td>
</tr>
<tr>
<td>mht1Δ mht1Δ (BY4742)</td>
<td>BY4742, Δyl062c::Kan' Δylp273w::Kan'</td>
<td>SGDp*</td>
</tr>
<tr>
<td>sam4Δ / mht1Δ</td>
<td>MATa Δylp273w::Kan' Δyl062c::Kan'</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Strains prepared by the Saccharomyces Genome Deletion Project (SGDp) and purchased from Invitrogen.

pooled, divided into 200-μl aliquots, and stored at −80 °C. [3H]AdoMet concentrations were calculated using a specific activity of 79,000 cpm/pmol provided by the manufacturer.

NMR Analysis of AdoMet Stereochemistry—AdoMet (chloride salt; purity of −70% with 1 mol/mol H2O and 4.6% methanol; Sigma) was dissolved in 0.1 M HCl. One aliquot was incubated at 37 °C for 160 h, whereas the other was not incubated. Both were then dried and dissolved in D2O to a final concentration of 10 mg/ml. The 1H NMR spectrum for 500 μl of each sample was determined using a Bruker ARX400 spectrometer operating at 400.13 MHz as described previously (27).

Isoaspartyl Protein Methyltransferase Assay—Human recombinant L-isoaaspartyl protein methyltransferase (0.13 μg, specific activity of 20,990 pmol/min/mg of protein) was incubated with 0.016 pmol of either (R,S)- or (S,S)-[3H]AdoMet in the presence or absence of 3.5 nmol of the isoaspartyl-containing peptide KASA(isoD)AKY (California Peptide Research, Inc., Napa, CA). The reaction was buffered in 50 mM BisTris-HCl (pH 6.4) in a final volume of 40 μl. After incubation for 45 min at 37 °C, methylation of the peptide was determined as described previously (30).

Yeast Strains—Table 1 lists the yeast strains used in this study. The sam4Δ and mht1Δ 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 sam4Δ / mht1Δ double knock-out strain was created by mating the MATa strain of sam4Δ with the MATa strain of mht1Δ 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 the non-parental ditype. The deletion of both the SAM4Δ and MHT1Δ genes was confirmed by PCR analysis using flanking TAG1 and TAG2 primers (www-sequence.stanford.edu/group/yeast_deletion_project/PCR_strategy.html).

Extract Preparation—Yeast extracts were prepared by modification of a previous method (31). Briefly, a single colony was inoculated in 6 ml of YPD medium (1% Bacto-yeast extract, 2% Bacto-peptone, and 2% dextrose) and incubated overnight with shaking at 30 °C. One ml of the culture was then transferred to 250 ml of YPD medium and grown to an absorbance of 1.0 at 600 nm. Cells were pelleted at 5000 × g for 5 min and washed twice with 5 ml of water. The cell pellet volume was estimated, and 2 volumes of lysis buffer (0.1 M sodium phosphate (pH 7.0) and 1 mM phenylmethylsulfonyl fluoride) and 1 volume of baked zirconium beads (BioSpec Products, Inc., Bartlesville, OK) were added. Cells were then lysed via six cycles of alternate 1-min periods of vortexing and incubating on ice. The lysate was removed from the beads and centrifuged at 14,000 × g for 50 min at 4 °C. The supernatant was then divided into aliquots of 500 μl and kept at −80 °C until needed. The total protein concentration for each lysate was determined using the method of Lowry et al. (32) after precipitation in 10% trichloroacetic acid and using bovine serum albumin as a standard.

Arabidopsis seed extract prepared as described previously (33) was provided by Sarah Villa (UCLA). Mouse liver and brain extracts were prepared as described previously (34) and provided by Jonathan Lowenson (UCLA). An extract of Caenorhabditis elegans prepared as described previously (35) was provided by Tara Gomez (UCLA). Drosophila embryonic extract prepared as described previously (36) was provided by Thomas Fellner (UCLA).

Homocysteine Methyltransferase Assays—A modification of the method of Shapiro et al. (37) was used. Unless indicated otherwise, 0.2 pmol of purified (R,S)- or (S,S)-[3H]AdoMet was incubated with or without the specified amount of n-homocysteine (Sigma) in 0.15 M sodium phosphate (pH 7.0) with −0.1 mg of extract protein in a total volume of 200 μl. The temperature of incubation was dependent on the type of extract used: 37 °C for mouse; 30 °C for yeast, Drosophila and C. elegans; and 40 °C for Arabidopsis. At the end of each reaction incubation, [3H]methionine was separated from [3H]AdoMet by cation-exchange chromatography on Dowex 50WX8-400 columns (0.5 cm, inner diameter, × 2 cm; Sigma). Prior to use, the Dowex resin was washed alternately with 1 M HCl and 1 M NaOH, each step being separated by an H2O wash. This washing sequence was repeated a second time, after which the resin was finally equilibrated with 0.1 M sodium phosphate (pH 7.0). After the reaction mixtures were allowed to flow through the columns, the columns were eluted with 2 ml of H2O, and the total effluent was collected in scintillation vials, mixed with 15 ml of Safety-Solve fluor, and counted on a Beckman LS6500 counter. The amount of [3H]methionine produced was calculated from this radioactivity using a specific activity of 79,000 cpm/pmol.

RESULTS

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 R,S form in vitro under physiological conditions to produce an S,S/R,S mixture that is roughly 50:50 (16–18). In Fig. 3, we compare the 1H NMR spectra of a commercial preparation of AdoMet with and without incubation to induce racemization, which was performed at pH 1.0 to minimize the formation of other AdoMet degradation products such as methylthioadenosine 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 isotypically labeled R,S and S,S forms by...
A. Non-isotopically labelled commercial AdoMet (no treatment)

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

FIGURE 3. 400 MHz $^1$H 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.

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.

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

FIGURE 5. Confirmation of the configuration of purified (S,S)- and (R,S)-[3H]AdoMet by their utilization by the protein l-isoaspartyl O-methyltransferase. Human recombinant l-isoaspartyl 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.

Cation-exchange HPLC (Fig. 4) (cf. Ref. 18). 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-isoaspartyl 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 mht1Δ 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 sam4Δ/mht1Δ extract and 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 sam4Δ 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 mht1Δ and sam4Δ 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 sam4Δ 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 mht1Δ 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 methyl donors, ranging from 32 to 44 $\mu$M, 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 $\mu$M, 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...
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.
homology to the two yeast gene products are the \( N^\circ \)-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-(\( \delta \)-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 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-L-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 in 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 *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

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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 methytransferase 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 methytransferases 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 methytransferases (50) and appear to represent a new class of methytransferases 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-aminoacyclopropane-1-carboxylate synthase usually reacts with (S,S)-AdoMet to produce 1-aminoacyclopropane 1-carboxylate and 5’-methylthriadenosine. However, with (R,S)-AdoMet, it can form 1-vinylglycine 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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Recognition of Age-damaged (R,S)-AdoMet

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