Methionine Metabolism and Liver Disease

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Abstract

In the early 1930s, Banting and Best, the discoverers of insulin, found that choline could prevent the development of fatty liver disease (steatosis) in pancreatectomized dogs treated with insulin. Later work indicated that in rats and mice, diets deficient in labile methyl groups (choline, methionine, betaine, folate) produced fatty liver and that long-term administration of diets deficient in choline and methionine also caused hepatocellular carcinoma. These experiments not only linked steatosis and diabetes but also provided evidence, for the first time, of the importance of labile methyl group balance to maintain normal liver function. This conclusion is now amply supported by the observation of mice devoid of key enzymes of methionine and folate metabolism and in patients with severe deficiencies in these enzymes. Moreover, treatments with various methionine metabolites in experimental animal models of liver disease show hepatoprotective properties.

Contents

SAMe: *S*-adenosylmethionine

METHIONINE METABOLISM

The first steps in mammalian methionine metabolism are conversion to *S*-adenosylmethionine (SAMe, abbreviated also as AdoMet) and transfer of the methyl group of SAMe to a large variety of methyl acceptors with formation of *S*-adenosylhomocysteine (SAH, abbreviated also as AdoHcy), an inhibitor of many SAMe-dependent methyltransferases (13, 19, 84). Although there are a large number of SAMe-dependent methyltransferases (19, 49, 59), the three reactions that quantitatively contribute most to the transmethylation flux are methylation of glycine by glycine *N*-methyltransferase (GNMT) to form sarcosine (*N*-methylglycine), methylation of guanidinoacetate by guanidinoacetate *N*-methyltransferase (GAMT) to form creatine, and methylation of phosphatidylethanolamine (PE) by PE *N*-methyltransferase (PEMT) to form phosphatidylcholine (PC) (70, 99). SAH is subsequently hydrolyzed to homocysteine (Hcy) and adenosine by SAH hydrolase (SAHH). Hcy is an important metabolic hub that can be remethylated to regenerate methionine, via either methionine synthase (MS, methyltetrahydrofolate-homocysteine methyltransferase) or betaine (trimethylglycine)-homocysteine methyltransferase (BHMT), or enter the transsulfuration pathway forming other products such as cysteine, taurine, glutathione (GSH), and sulfate, converted to homocysteine thiolactone, or can be excreted to the plasma (100). Finally, SAMe can also regenerate methionine via methyl-thioadenosine (MTA), a by-product of polyamine synthesis (**Figure 1**). The relevant pathways of methionine metabolism are summarized in **Figure 1**. Although all mammalian cells can synthesize and utilize SAMe, the completed methionine cycle, as depicted in **Figure 1**, is most prominent in the liver, kidney, and pancreas (29).

In the liver, when the total consumption of methyl groups through the transmethylation pathway is more than the dietary intake of labile methyl groups (from methionine, choline, and betaine), the difference is made up by de novo synthesis of labile methyl groups via the methylneogenesis pathway: the formation of methyltetrahydrofolate (methyl-THF) from

methylene-THF by methylene-THF reductase (MTHFR) and the subsequent transference of the methyl group of methyl-THF to Hcy via MS to form methionine (**Figure 1**) (70, 99). SAMe regulates the balance between these two pathways. This was first proposed by Finkelstein et al. (30, 31) on the basis of their findings that SAMe is an allosteric activator of CBS and on the earlier observation by Kutzbach & Stokstad (53, 54), confirmed later by Jenks & Matthews (46), demonstrating that SAMe is an allosteric inhibitor of MTHFR. SAMe is also an allosteric activator of GNMT (76) and of methionine adenosyltransferase (MAT) III (11, 103), the predominant liver isoenzyme that synthesizes SAMe (51), which in addition is activated by methionine (93). Thus, SAMe functions as a methionine balance gauge: When the availability of methionine is low, hepatic SAMe content drops, releasing the inhibition this molecule exerts on the synthesis of methionine through the methylneogenesis pathway so that normal levels of methionine can be restored. Conversely, when the concentration of methionine is elevated, hepatic SAMe content increases, causing the activation of methionine catabolism via the transmethylation and transsulfuration pathways and the inhibition of methionine regeneration via the methylneogenesis pathway, thus restoring normal methionine content (**Figure 1**). In addition to this role of SAMe as a methyl balance regulator, SAMe stabilizes CBS against proteolysis (85), upregulates the expression of *MAT1A* (34), the main gene responsible of hepatic SAMe synthesis (51), and inhibits the expression of *BHMT* (78), which indicates that SAMe is also a long-term regulator of hepatic methionine metabolism.

The main features of hepatic one-carbon metabolism (**Figure 1**) are redundancy of the pathways that regenerate methionine and metabolize Hcy together with a tight metabolic control by SAMe of the folate and methionine cycle and of the transsulfuration pathway, a potent feedback inhibition by SAH of the transmethylation pathway, and a saving effect of methyl-THF on SAMe consumption. In recent years, one-carbon metabolism has received a great deal of attention because disruption of methyl balance in a variety of genetically modified mice is associated with the development of various forms of liver injury, namely fatty liver disease and HCC, and because patients with liver disease often have abnormal expression of key genes involved in methionine metabolism as well as elevated serum levels of methionine and Hcy. We review here the lines of evidence linking abnormal methionine metabolism and liver disease.

METHIONINE ADENOSYLTRANSFERASE DEFICIENCY AND LIVER DISEASE

Two MAT isoenzymes, MATI and MATIII, are expressed primarily in the adult liver. They are, respectively, tetramers and dimers of the same single subunit encoded by the gene *MAT1A* (51). A third isoenzyme, MATII, contains a catalytic subunit encoded by a second gene, *MAT2A*, and is expressed in all tissues including adult liver, although to a smaller extent (51). MATI, MATII, and MATIII differ in their kinetic and regulatory properties and, consequently, methionine metabolism and cellular SAMe content varies drastically depending on the type of enzyme that is expressed. MATI and MATII both have low K_m for methionine but SAMe inhibits only MATII; MATIII has high K_m for methionine and is activated by both SAMe and methionine (11, 51, 79, 103). Consistent with this tissue distribution and kinetics, individuals deficient in hepatic MATI/III activity due to mutations of the *MAT1A* gene have persistent hypermethioninemia with normal plasma levels of total Hcy (tHcy) (15, 106). A similar situation is observed in *MAT1A*-knockout mice (60). In these mutant mice, the hepatic expression of *MAT2A* is markedly induced to compensate for the loss of MATI/III; however, since MATII does not have the same high catalytic capacity as that of MATI/III, *MAT1A*-knockout mice have elevated plasma levels of methionine and reduced hepatic SAMe and GSH content (60).

SAH: S-adenosylhomocysteine

GNMT: glycine *N*-methyltransferase

PEMT: phosphatidylethanolamine *N*-methyltransferase

SAHH: S-adenosylhomocysteine hydrolase

MS: methyltetrahydrofolate-homocysteine methyltransferase or methionine synthase

BHMT: betaine-homocysteine methyltransferase

Transsulfuration pathway: the group of reactions that start with the conversion of the sulfur of methionine into the sulfur of cysteine and that lead to the synthesis of a variety of sulfur-containing molecules including glutathione, taurine, and sulfate

Methionine cycle: the conversion of methionine into homocysteine via S-adenosylmethionine and S-adenosylhomocysteine and the regeneration of methionine by methylation of homocysteine

Transmethylation pathway: the transfer of the methyl group of SAMe to a large variety of methyl acceptors with formation of S-adenosylhomocysteine

Labile methyl groups: methyl groups, such as those in methionine, choline, betaine, and folate, that can be transferred to other acceptor molecules

MTHFR: methylenetetrahydrofolate reductase

CBS: cystathionine β-synthase

MAT: methionine adenosyltransferase

Fatty liver disease: a clinicopathological term that encompasses a spectrum of abnormalities ranging from simple triglyceride accumulation in the hepatocytes (steatosis) to hepatic steatosis with inflammation (steatohepatitis)

One-carbon metabolism: the group of reactions comprising the methionine and folate cycle

MAT2A expression associates with liver proliferation and carcinogenesis, whereas *MAT1A* expression diminishes during liver regeneration and after partial hepatectomy, and is inhibited in HCC (66, 67). Moreover, the expression of *MAT1A* in rat hepatoma cells results in the inhibition of cell growth (12), and the administration of SAMe to hepatoma cells induces cell death via apoptosis (117). Consistent with these observations, livers from *MAT1A*-knockout mice exhibit increased expression of genes involved in hepatocyte proliferation (60), abnormal liver regeneration after partial hepatectomy (16), are more sensitive to developing steatosis in response to a methionine- and a choline-deficient diet (60), and spontaneously develop steatohepatitis (liver steatosis with inflammation) (60) and HCC (63).

Unlike mice, individuals with *MAT1A* mutations—more than 30 such subjects carrying out 17 different mutations have been described—have normal liver function (15, 106), which suggests that human liver is more resistant than rodent liver to an imbalance in methyl group metabolism caused by MATI/III deficiency. Patients with liver cirrhosis, however, have abnormal methionine metabolism (41), reduced *MAT1A* expression (5), and MAT activity (26). Moreover, a causal relationship between MATI/III deficiency and liver disease is suggested by the beneficial effect of treatment with SAMe on survival in patients with alcoholic liver cirrhosis (65). These results indicate that although in humans a deficiency in hepatic MATI/III alone may not be sufficient to trigger liver injury, this condition may facilitate the initiation and progression of liver disease by other causes such as excessive ethanol consumption, viral infection, or obesity.

Several young individuals with *MAT1A* mutations leading to severe impairment of MAT1/III activity have developed neurological disorders including, in two cases, brain demyelination (15). One of these two cases was treated with SAMe, and brain demyelination was reversed (15). These findings raise the issue of how deficient hepatic SAMe synthesis affects brain function and why only some subjects with mutations in *MAT1A* develop brain demyelination. The subjects showing demyelination of the brain express truncated forms of the subunit encoded by *MAT1A* (15). We hypothesize that subunit interaction between truncated mutant *MAT1A* subunits and normal *MAT2A* subunits will prevent the production of active MATII enzymes, further aggravating hepatic SAMe synthesis in subjects carrying out these mutations. Although there is no evidence showing negative interaction between truncated forms of *MAT1A* and normal *MAT2A* subunits, the amino acid sequence of *MAT1A* and *MAT2A* subunits is very similar (51), and negative interaction between mutant *MAT1A* subunits has been suggested to explain hypermethioninemia (*a*) in two young children who are compound heterozygous for two mutations in *MAT1A* (50) and (*b*) between a mutant and a normal *MAT1A* subunit to explain the dominant inheritance of hypermethioninemia in subjects carrying a R264H mutation in *MAT1A* (82). Moreover, there is a lack of information at present about how a reduction in hepatic SAMe synthesis may affect brain myelin synthesis.

TRANSMETHYLATION DEFICIENCY AND LIVER DISEASE

Phosphatidylethanolamine *N*-methyltransferase Deficiency

PEMT is a liver-specific enzyme that converts PE into PC via three successive *N*-methylations of the ethanolamine moiety of PE using SAMe as the methyl donor. Approximately one-third of liver PC in mice is synthesized by this pathway, and the remainder is generated by the cytidine-diphosphate-choline (CDP-choline) pathway, which does not consume SAMe (25, 88). These two pathways, which are functionally different (42, 43), produce different profiles of PC species, being PC molecules synthesized by the methylation pathway more polyunsaturated than those produced by the CDP-choline pathway (25). Thus, overexpression of *PEMT* cannot compensate for

impaired PC synthesis via the CDP-choline pathway in CHO cells with a temperature-sensitive mutation in the CDP-choline pathway, and die, via apoptosis, above the restrictive temperature (43). Similar to the situation observed with the hepatic expression of *MAT1A* and *MAT2A*, the expression of the CDP-choline pathway associates with liver proliferation and carcinogenesis, whereas *PEMT* expression diminishes during liver regeneration and after partial hepatectomy, and is inhibited in HCC (24, 42).

PEMT-knockout mice fed a normal diet have been reported to have normal (111) or moderate fatty liver (119) and, when fed a choline-deficient diet, develop steatohepatitis and liver failure within days due to loss of membrane integrity caused by a reduced PC/PE ratio (58, 112, 119). This may be clinically important since patients with liver cirrhosis have reduced hepatic PEMT activity (26). A genetic *PEMT* variant that results in an amino acid substitution (V175M) and reduced PEMT activity has been shown to occur more frequently in subjects with NAFLD than in a control group with normal liver (97). Although this study was carried out in a small group of subjects, this finding further suggests that reduced PEMT activity is a risk factor for liver steatosis. Triglycerides are synthesized in the liver and then secreted in very-low-density lipoproteins (VLDLs). The formation of VLDLs requires the synthesis of new PC molecules, and when these are not available, lipid droplets accumulate in the cytosol of hepatocytes, causing steatosis. Accordingly, a reduction in PEMT activity may facilitate the development of liver steatosis, particularly if it interacts with other conditions, such as a dietary deficiency in choline that will limit the availability of CDP-choline, a reduced synthesis of methionine via the methylneogenesis pathway, or an impaired synthesis of SAMe.

PEMT-knockout mice have reduced plasma tHcy, whereas the concentration of plasma methionine, cystathionine, and cysteine are unchanged (70, 75). In mutant mice, liver MAT, MTHFR, BHMT, MS, and CBS activities are normal (49), as are the hepatic concentration of methionine, SAMe, SAH, and tHcy (70). Plasma sarcosine is elevated in the plasma of *PEMT*-knockout mice (70), which suggests that the reduction in total transmethylation flux caused by the absence of PEMT is compensated by GNMT. These results suggest that the reduced content of plasma tHcy in *PEMT*-knockout mice is independent of the liver content of SAMe, SAH, or tHcy and may be the result of altered transport due to abnormal membrane phospholipid composition.

Glycine *N*-methyltransferase Deficiency

GNMT is the most abundant methyltransferase in mammalian liver, comprising about 1% of the total soluble protein in rat liver (76). GNMT is also present in large amounts in the pancreas and the prostate. GNMT catalyzes the conversion of glycine into sarcosine, which is then oxidized to regenerate glycine. The function of this futile cycle is to catabolize excess liver SAMe to maintain a constant SAMe/SAH ratio and avoid aberrant methylation reactions (70, 99). Accordingly, individuals with *GNMT* mutations that lead to inactive forms of the enzyme have elevated plasma levels of methionine and SAMe, but the concentration of tHcy is normal (4, 71); the liver of *GNMT*-knockout mice show a marked increase in the concentration of SAMe and methionine, but the content of SAH is normal (61). These findings indicate that the hepatic reduction in total transmethylation flux caused by the absence of GNMT cannot be compensated by other methyltransferases that are abundant in the liver, such as PEMT and GAMT, and that this situation leads to the accumulation of hepatic SAMe and increased transport of this molecule to the plasma.

Although individuals with mutations of *GNMT* have mild to moderate liver disease with elevated serum transaminases, they appear to be normal with the exception of an abnormal pattern of plasma serum methionine metabolites (4, 71). However, these individuals were quite young and liver disease may progress as they

get older. The finding that *GNMT*-knockout mice have elevated serum transaminases (57a, 63a) and spontaneously develop steatosis and HCC (63a) supports this hypothesis. As it occurs with *MAT1A* and *PEMT* expression, *GNMT* is absent in HCC (17) and is downregulated in the liver of patients at risk to develop HCC, such as in hepatitis C virus- and alcohol-induced liver cirrhosis (5). Two polymorphisms located in the promoter region of *GNMT* that cause reduced expression of the gene have been identified (105). Loss of heterozygosity of these two polymorphisms is higher in DNA isolated from the tumor than in DNA isolated from liver adjacent to the tumor (105), which suggests that reduced GNMT activity may be an early event in HCC development.

Guanidinoacetate *N*-methyltransferase Deficiency

GAMT is the enzyme that catalyzes the last step of creatine synthesis. The enzyme is found in abundance in the liver, kidney, skeletal muscle, cardiac tissues, and pancreas, and in smaller amounts in all brain areas (94). GAMT deficiency is an inborn error of creatine biosynthesis, characterized by accumulation of guanidinoacetic acid and depletion of creatine, that responds to treatment with oral creatine supplementation (101). A variety of neurological symptoms, including muscular hypotonia and weakness, poor head control, neurodevelopmental delay, extrapyramidal movement disorders, epilepsy, and autistic or self-aggressive behavior in older patients have been described in patients with GAMT deficiency (101). Although *GAMT*-knockout mice have reduced body weight and male mice have reduced fertility, they have only moderate cognitive disorders (94) and appear to be normal with the exception of abnormal levels of serum guanidinoacetic acid and creatine. Serum levels of methionine, SAMe, and tHcy are normal in GAMT-deficient patients, whereas sarcosine content is elevated (70), which suggests that the reduction in total transmethylation flux caused by the absence of GAMT is compensated by GNMT and that this genetic defect has little impact on total labile methyl group balance.

S-ADENOSYLHOMOCYSTEINE HYDROLASE DEFICIENCY AND LIVER DISEASE

SAHH is a ubiquitous enzyme that catalyzes the hydrolysis of SAH to adenosine and Hcy (**Figure 1**). This reaction is reversible and, although the synthetic direction is thermodynamically favored, under normal conditions the removal of both adenosine and Hcy is sufficiently rapid to maintain the flux in the direction of hydrolysis (**Figure 1**). Adenosine can be metabolized by adenosine deaminase (ADA) or adenosine kinase (ADK). After birth, ADK is believed to be the primary route of adenosine metabolism in the liver (9). Blocking the removal of adenosine by disruption of the *ADK* gene in mice results in an elevation of hepatic SAH and SAMe content (9). *ADK* mutant mice developed severe liver steatosis and died about eight days after birth (9), although the SAMe/SAH ratio remained normal. During embryogenesis, ADA is the major adenosine-metabolizing enzyme. As expected, *ADA*-knockout mice have elevated hepatic SAH and SAMe levels and a reduced SAMe/SAH ratio (68, 110). These mutant mice die in the embryonic state with severe liver necrosis. These results stress the importance of the removal of excess adenosine to keep intracellular SAH content within a certain physiological range to maintain activity in the transmethylation pathway. As mentioned above, three enzymes (MS, BHMT, and CBS) metabolize Hcy in the liver. Mouse models defective in each of these three enzymes have been generated, and although all have increased plasma tHcy, they differ markedly in the degree by which the hepatic SAMe/SAH ratio and liver function are affected (see below).

Three individuals with mutations in the *SAHH* gene leading to very-low-activity forms of the enzyme have been identified (7, 8, 10). These subjects have severe myopathy, developmental delay, and elevated serum creatine

kinase and transaminases. In *SAHH*-deficient patients, serum SAH and SAMe content is markedly elevated, the ratio SAMe/SAH is reduced, and tHcy is normal or slightly elevated (7, 8, 10). Serum PC and choline content is low, which may be the result of a reduced biosynthesis of PC by methylation of PE, a process that is inhibited by SAH (7, 8, 10). Liver biopsies in two of the three patients presented signs of chronic hepatitis and moderate fibrosis (8, 10). Methionine restriction, together with creatine and PC supplementation, improved myelination and psychomotor development in these patients (7, 8, 10).

The mouse *lethal nonagouti* mutation has been shown to delete the *SAHH* gene (69). These mutant mice die between the late blastocyst and early implantation stages (69). However, these studies should be taken with caution since additional genes are removed by this deletion, and the embryonic lethality observed in these mice at the preimplantation stage cannot be attributed with certainty to a complete deficiency of SAHH activity (69). Generation of *SAHH*-knockout mice will be of great value to better understand the role of SAHH deficiency in liver pathophysiology.

HOMOCYSTEINE METABOLISM DEFICIENCY AND LIVER DISEASE

Methionine Synthase Deficiency and Methionine Synthase Reductase Deficiency

MS is a ubiquitous enzyme that connects the folate cycle with methionine metabolism (**Figure 1**). MS catalyzes the methylation of Hcy to generate methionine, using methyl-THF as the methyl group donor, and requires the presence of an enzyme-bound cobalamin (vitamin B_{12}) prosthetic group for activity (**Figure 1**). The reaction involves the transference of the methyl group of methyl-THF to the enzyme-bound cobalamin to form methylcobalamin, and the subsequent transference of the methyl group from methylcobalamin to Hcy to generate methionine (6). Every 2,000 turnovers, the highly reactive cob(I)alamin cofactor of MS is oxidized to the inert cob(II)alamin form, which inactivates the enzyme. MS is then reactivated through reductive methylation to regenerate methylcobalamin using SAMe as the methyl donor in a reaction catalyzed by the enzyme methionine synthase reductase (MSR) (**Figure 2**) (6, 77, 116). Because MS is the only mammalian enzyme that uses methyl-THF, MS deficiency or MSR deficiency creates a folate trap that accumulates cellular folate as methyl-THF (**Figure 1**) and makes folate unavailable for other folate-dependent reactions, such as purine and pyrimidine biosynthesis. Patients with *MS* or *MSR* deficiencies have homocysteinemia and homocystinuria but low levels of methionine. Megaloblastic anemia with or without some degree of neurological symptoms (cerebral atrophy and alterations in muscle tone) and mental retardation are common in these patients (14). Liver symptoms

Folate cycle: the set of reactions linking tetrahydrofolate, methylene-tetrahydrofolate, and methyl-tetrahydrofolate

Folate trap: the accumulation of cellular folate as methyl-tetrahydrofolate

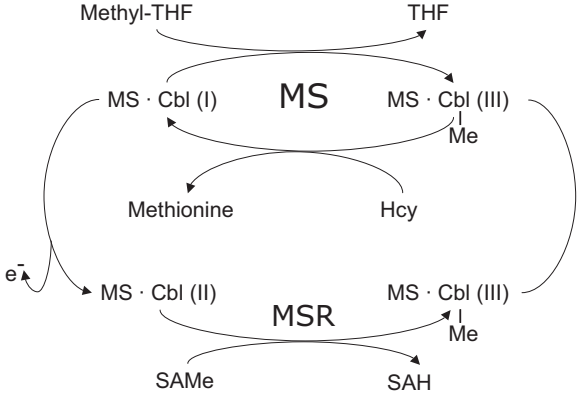

Figure 2

Methionine synthase (MS) catalyzes the methylation of homocysteine (Hcy) to generate methionine, using methyl-tetrahydrofolate (methyl-THF) as the methyl group donor. MS requires the presence of an enzyme-bound cobalamin (Cbl, vitamin B_{12}) prosthetic group for activity. The reaction involves the transference of the methyl group of methyl-THF to the enzyme-bound Cbl(I) to form methylcobalamin [MeCbl(III)], and the subsequent transference of the methyl group from MeCbl(III) to Hcy to generate methionine. Every 2,000 turnovers, the highly reactive Cbl(I) cofactor of MS is oxidized to the inert Cbl(II) form, which inactivates the enzyme. MS is then reactivated through reductive methylation to regenerate MeCbl(III) using SAMe as the methyl donor in a reaction that is catalyzed by the enzyme methionine synthase reductase (MSR) and that generates SAH.

Gene trap: an approach that randomly disrupts genes through the genome by inserting a DNA element (usually containing a reporter). The disruption usually mutates the endogenous gene

have not been reported in patients with *MS* or *MSR* deficiency.

MS-knockout mice embryos survive through implantation but die soon thereafter, which indicates the importance of this reaction in early development and suggests that *MS*-deficient patients may have residual MS activity or there is some compensatory mechanism that is absent in mice (104). A mouse model with a gene trap insertion in the *MSR* gene has been generated (27). As anticipated, these mice have reduced MSR and MS activity, thus avoiding the expected lethality associated with the complete absence of MS activity (27). *MSR*-deficient mice have increased plasma tHcy and reduced plasma methionine (27). SAMe level is higher in the liver of *MSR*-deficient mice than in wild-type animals, and SAH content is lower in the mutant mice (27). Animals with *MSR* deficiency accumulate cellular folate as methyl-THF (**Figure 1**). Methyl-THF binds and inactivates GNMT, which could explain the elevation in hepatic SAMe and increased SAMe/SAH ratio observed in *MSR*-knockout animals just as it occurs in *GNMT*-deficient mice. In other tissues, like brain and kidney, SAMe and SAH content is normal in *MSR*-deficient mice, whereas in the heart, the expected trend is observed with lower levels of SAMe, increased SAH content, and a slightly reduced SAMe/SAH ratio (27). Megaloblastic anemia, a common symptom in patients with *MSR* mutations, and abnormal liver function have not been observed in *MSR*-deficient mice (27).

Methylenetetrahydrofolate Reductase Deficiency and Liver Disease

MTHFR catalyzes the reduction of methylene-THF, the donor of a one-carbon group for deoxythymidine-monophosphate (dTMP) synthesis, to methyl-THF, the methyl donor for the methylation of Hcy to methionine in the reaction catalyzed by MS (**Figure 1**). Severe *MTHFR* deficiency is the most common inborn error of folate metabolism (72). The major laboratory findings are high serum tHcy and low methionine. The predominant clinical findings are neurological and thrombosis, although fatty liver also occurs frequently (91). Because *MTHFR* deficiency does not lead to methylene-THF deficiency, patients with *MTHFR* deficiency do not develop megaloblastic anemia (91). A knockout mouse model deficient in *MTHFR* has been generated (18). Homozygous knockout mice show a variable phenotype with reduced survival, suggesting a role for *MTHFR* during early development (18). *MTHFR*-knockout mice are smaller than wild-type animals and show developmental retardation with cerebellar pathology (18). As expected, *MTHFR*-deficient mice have elevated levels of tHcy (about tenfold) and decreased content of methionine (about twofold) in plasma (18, 36). In a variety of tissues, including the liver, the levels of methyl-THF and SAMe are decreased, the content of SAH is increased, the SAMe/SAH ratio is reduced, and global DNA is hypomethylated (18, 36, 95). Moderate lipid deposition in the proximal portion of the aorta has been observed in *MTHFR*-knockout mice at 14 months of age (18), although the lesion is less advanced than in other well-documented models for atherosclerosis, such as the *apoE*-knockout mouse (87). *MTHFR*-knockout mice develop severe liver steatosis (18, 95), a condition seen also in patients with severe *MTHFR* deficiency (72). The administration of betaine prevented or ameliorated the development of steatosis and reduced plasma tHcy levels in *MTHFR*-knockout mice, but had little impact on plasma methionine (95). These findings indicate that in mice fed a normal diet, the reduction in hepatic methylneogenesis, caused by the absence of MTHFR, cannot be compensated by the BHMT pathway unless animals are supplemented with betaine, and that this situation leads to high serum tHcy and low methionine with a concomitant reduction in the hepatic SAMe content and a reduced SAMe/SAH ratio. Moreover, these results suggest that individuals with moderate hyperhomocysteinemia, due to a deficiency in *MTHFR*, may have a higher risk of developing fatty liver. The *MTHFR* C677T polymorphism,

a common variant that is associated with diminished enzymatic activity (33), has been linked consistently with steatosis and fibrosis in patients with chronic hepatitis C (2).

The C-to-T transition at nucleotide 677 (C677T) of the *MTHFR* gene results in an alanine-to-valine change in the protein that is associated with diminished enzymatic activity and mild hyperhomocysteinemia, particularly when folate status is low (33). This variant, with lower enzymatic activity, associates with an increased risk for neural tube defects and pregnancy complications (91). Many studies have reported that this variant is also a risk factor for vascular disease (91). Individuals homozygous for this mutation (10%–15% of Caucasians) who maintain adequate folate levels have normal blood tHcy levels, as folate may stabilize the enzyme and allow it to function normally (37). Another polymorphism, an A-to-C transition located at nucleotide 1298 (A1298C), results in a glutamate-to-alanine change in the protein that is also associated with diminished enzymatic activity and increased tHcy, but to a lesser extent than the C677T mutation (114). Epidemiologic studies have noted that the C677T and/or A1298C genetic variants are in linkage disequilibrium, and that both genotypes are associated with a reduced risk of colorectal carcinoma and HCC (62, 92, 118).

Thymidylate synthase (TYMS) catalyzes the synthesis of dTMP from deoxyuridine-monophosphate, a process that requires methylene-THF as the donor of a one-carbon group (**Figure 1**). Consequently, genetic variants that lead to low hepatic MTHFR activity and increased TYMS activity will result in an increased flow of methylene-THF molecules into dTMP synthesis, thus minimizing the misincorporation of uracil moieties into DNA and the occurrence of DNA double-strand breaks, which may explain the reduced risk of HCC in subjects carrying this genotype (118). However, although reduced hepatic MTHFR activity channels folate cofactors to DNA synthesis and repair, which reduces the risk of developing HCC, reduced MTHFR activity also induces a decrease in the hepatic content of SAMe and of the SAMe/SAH ratio, which inhibits methylation reactions, including DNA and histone methylation, and increases the risk for liver injury, including HCC. A balance between these two critical pathways (DNA repair and methylation reactions) must be reached so that neither is compromised and the risk of developing liver injury is minimized (see Summary section).

Folate Transporter Deficiency and Liver Disease

Folate is a term used to refer to a series of compounds derived from THF (**Figure 1**). Serum tHcy levels show a significant inverse correlation with serum folate concentration, and folate fortification of the diet and supplement use of this vitamin often reduce tHcy levels (91). The liver contains the highest percent of body folate, and the level of folate-dependent enzymes is also high. Folate is transported from the extracellular space to the cytoplasm of the cell mainly via either the reduced folate carrier 1 (*RFC1*) or the folate-binding protein 1 (*FOLBP1*) (96, 98). RFC1 is an anion exchanger that mediates folate delivery into many cell types and has a high affinity for reduced folates, such as methyl-THF, whereas FOLBP1 is a glycosylphosphatidylinositol anchored protein that transports folic acid and methyl-THF with high affinity (96, 98). Homozygous *FOLBP1*-knockout mice are embryonic lethal but may be rescued by supplementing pregnant heterozygous mice with folate, and pups do not require further folate supplementation (83). Homozygous *RFC1*-knockout mice are also embryonic lethal; however, supplementation with folate does not yield viable offspring (83). When heterozygous *FOLBP1*-knockout mice are maintained on a reduced–folic acid diet, the plasma concentration of tHcy is markedly increased (83). Folate transport gene inactivation in mice has been shown to increase the sensitivity to colon cancer. It will be interesting to analyze whether the inactivation of any of these two folate

transporters affects liver function or makes the liver more sensitive to injury. These studies are of particular interest because folate deficiency is one of the most common nutritional abnormalities in patients with chronic alcoholism, especially in those who have developed alcoholic liver injury (23). Folate deficiency disturbs hepatic methionine metabolism and makes the liver more prone to injury in response to a variety of hepatotoxic agents, including alcohol (38).

Betaine-Homocysteine Methyltransferase Deficiency and Liver Disease

Betaine is an intermediate of choline oxidation (**Figure 1**), and the enzymes of this pathway are primarily found in the liver and kidney of mammals (27). The physiologic function of betaine is either as an organic osmolite, to protect cells under stress (27), or as a source of methyl groups for the formation of methionine from Hcy in a reaction catalyzed by BHMT (**Figure 1**). *BHMT* is expressed mainly in the liver and kidney (80). Inhibition of BHMT in mice, with the specific inhibitor *S*-(δ-carboxybutyl)-DL-homocysteine, causes an increase in plasma tHcy and a reduction in the liver of both SAMe content and the SAMe/SAH ratio (21). These findings indicate that MS cannot compensate the hepatic reduction in total formation of methionine from Hcy caused by the inhibition of BHMT. Whether long-term inhibition of BHMT induces fatty liver or other forms of liver injury is not known at present. In rats, betaine administration prevents liver injury induced by a variety of toxins including carbon tetrachloride, alcohol, and lipopolysaccharide (22); in humans, dietary betaine has been shown to improve liver function in nonalcoholic fatty liver disease, including steatohepatitis (1). These findings suggest that chronic BHMT deficiency may facilitate the initiation and/or progression of liver disease in animal models as well as in humans.

Cystathionine β-Synthase Deficiency and Liver Disease

CBS catalyzes the condensation of Hcy and serine, thereby forming cystathionine, which is subsequently cleaved to cysteine. CBS is also the first reaction connecting the methylation cycle with the transsulfuration pathway (**Figure 1**). The major cause of homocystinuria is mutation of the gene encoding CBS. More than 130 different *CBS* mutations have been reported, with the mutation C833T the most frequently observed (52, 56). Deficiency of CBS activity results in elevated levels of tHcy and methionine in urine and plasma and decreased content of cystathionine and cysteine (32, 55, 70). Mental retardation, dislocation of the ocular lenses, convulsive tendencies, thromboembolic episodes, cardiovascular disease, and hepatic steatosis are common in these patients (32, 55, 72).

Homozygous *CBS* knockout mice rarely survive after four weeks of age, and the surviving animals show plasma tHcy levels above 200 μM, which are similar to those observed in patients with severe CBS deficiency (11). These mutant mice also have elevated levels of hepatic SAH, decreased SAMe/SAH ratio, and DNA hypomethylation (73, 89, 90, 113). *CBS*-knockout mice have abnormal lipid metabolism and spontaneously develop fatty liver disease, including steatohepatitis (73, 89, 90, 113).

SAMe, FOLATE, AND BETAINE IN THE TREATMENT OF LIVER DISEASE

In summary, the available evidence indicates that genetic disruption of methionine or folate metabolism in mice often leads to liver disease, mainly fatty liver and HCC; in humans, genetic mutations that severely impair one-carbon metabolism are also often associated with liver injury. The importance of impaired methionine metabolism in liver disease and the role that genetic polymorphism may play in determining the risk factor for liver

disease and cancer is illustrated by the observation that patients with liver disease of various etiologies (namely alcoholic and/or viral cirrhosis) frequently show an impaired clearance of methionine after an oral load of this amino acid (41). These patients also have elevated plasma tHcy (5, 22), reduced hepatic MAT and PEMT activity (26), and diminished expression of a variety of genes involved in methionine metabolism, such as *MAT1A*, *GNMT*, *MS*, and *BHMT* (5). In addition, findings link the loss of *GNMT* expression with HCC development (17, 105) and the reduced risk of HCC in subjects carrying specific variants of *MTHFR* and *TYMS* (118).

Ample evidence supports a role of SAMe, folate, and betaine in the treatment of liver disease (86). SAMe supplementation may attenuate liver injury by decreasing oxidative stress induced by CYP2E1 activation, restoring the cellular energy balance via regulation of AMP-activated protein kinase (AMPK), reducing inflammation through the downregulation of TNF-alpha and the upregulation of interleukin-10 synthesis, increasing the SAMe/SAH ratio, restoring normal methylation reactions, and inhibiting the apoptosis of normal hepatocytes while stimulating the apoptosis of liver cancer cells (3, 40, 64, 74, 81, 108, 117). Folate and betaine may attenuate liver injury by increasing hepatic SAMe synthesis, decreasing the hepatic content of Hcy and SAH, and increasing the SAMe/SAH ratio, which can restore normal methylation of DNA, proteins, and small molecules such as PE (86). SAMe's effect on growth, apoptosis, and TNF-alpha expression can be mimicked by MTA (3, 40, 81, 108). MTA inhibits methylation reactions and polyamine synthesis and can be metabolized to regenerate methionine (20) (**Figure 1**). The mechanism by which MTA attenuates liver injury is not clear at present but deserves further investigation. In conclusion, these findings point to one-carbon metabolism as a crucial therapeutic target in liver disease, particularly in the development of fatty liver and HCC.

Riboswitches: structural domains embedded within the noncoding sequences of certain mRNA that serve as metabolic-response genetic control elements

Role of SAMe

SAMe-binding proteins have been identified with functions that vary from methylation of DNA, histones and other proteins, phospholipid and small molecules such as arsenic, to the synthesis of polyamines, radical formation, and binding to mRNA riboswitches (**Figure 3**). SAH is an inhibitor of many SAMe-dependent methyltransferases (19). Because mice models with disruption of one-carbon metabolism have abnormal hepatic SAMe, SAH, or SAMe/SAH ratios (**Table 1**), aberrant hepatic methylation of DNA and histones is likely to occur. Methylation of DNA and of specific histone lysine residues plays a crucial role in the control of gene activity and nuclear architecture (28, 102). The emerging model is that a specific combination of histone modifications (namely methylation and

Table 1 Disrupted one-carbon metabolism genes and effect on liver SAMe, SAH, and SAMe/SAH ratio, and on plasma methionine and total homocysteine

Gene	SAMe	SAH	SAMe/SAH	Methionine	tHcy
MAT1A	Decrease	Unchanged	Decrease	Increase	Unchanged
GNMT	Increase	Unchanged	Increase	Increase	Unchanged
CBS	Decrease	Increase	Decrease	Decrease	Increase
MTHFR	Decrease	Increase	Decrease	Decrease	Increase
ADK	Increase	Increase	Unchanged	nd	nd
ADA	Increase	Increase	Decrease	nd	nd
MSR	Increase	Decrease	Increase	Unchanged	Increase
PEMT	Unchanged	Unchanged	Unchanged	Unchanged	Decrease

nd, not determined.

acetylation) together with DNA methylation regulates chromatin structure and gene function (28, 102). We hypothesize that the disorganization of the normal pattern of epigenetic modifications may be central to liver disease induced by disruption of one-carbon metabolism. To identify the essential pattern of epigenetic modifications that is altered in mice devoid of key enzymes of methionine and folate metabolism and that lead to liver injury, it is crucial to obtain genomewide information about DNA methylation as well as histone-modification maps from liver samples of the various mice models available. Ultimately, this approach will provide us with a catalog of genes silenced or activated in liver injury that could be validated in liver samples from patients with hepatic disease.

Altered hepatic methionine metabolism will likely lead to abnormal methylation of other key cellular proteins. Incubation of mice hepatocytes with SAMe induces the methylation of the phosphoprotein phosphatase 2A (PP2A) catalytic subunit (M.L. Martínez-Chantar, S.C. Lu, & J.M. Mato, unpublished observations), which promotes its functional association with regulatory subunits and activation (57). PP2A is one of the four major classes of eukaryotic serine/threonine phosphoprotein phosphatases, which affects such diverse cellular processes as metabolism, signal transduction, cell cycle progression, apoptosis, transcription, DNA replication, and protein synthesis (109). Alteration of the normal methylation status of PP2A, by changes in the concentration of SAMe, SAH, or the ratio SAMe/SAH, may be key in explaining the multiple effects in the liver caused by the disruption of one-carbon metabolism, as well as in explaining the mechanism by which SAMe, MTA, betaine, and folate prevent liver injury (see below).

The domain in the enzyme CBS that binds SAMe, known as the CBS domain, is widespread in nature; in humans there are approximately 50 proteins with this domain (see **http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=pfam00571** for an updated list of proteins with CBS domains). Although the precise function of the CBS domains in many of these proteins remains to be elucidated, the importance of CBS domains in disease is emphasized by the finding that point mutations are responsible for several hereditary diseases, such as homocystinuria (mutations in CBS), familial hypertrophic cardiomyopathy (mutations in AMPK), retinitis pigmentosa (mutations in inosine 5′-monophosphate dehydrogenase), and myotonia, idiopathic generalized epilepsy, Dent's disease, osteopetrosis, and Bartter syndrome (mutations in chloride channels, CLC) (44). CBS domains function as metabolic sensors by binding SAMe (in the case of CBS), AMP (in AMPK), or ATP (in inosine 5′-monophosphate dehydrogenase and CLC). Whether in addition to the enzyme CBS other mammalian proteins with CBS domains bind SAMe is not known at present, but the question merits a detailed investigation.

AMPK is regulated by the cellular AMP concentration and the AMP/ATP ratio. AMPK is activated in response to ATP depletion caused by stress—such as glucose deprivation, hypoxia, ischemia, and heat shock—and regulates cellular metabolism by inhibiting ATP consuming pathways—such as fatty acid and cholesterol synthesis—and activating pathways that generate ATP glycolysis and fatty acid oxidation (39). As such, AMPK behaves as a control switch to restore energy homeostasis. The function of AMPK, however, goes beyond cell energy homeostasis and has other physiological roles, such as regulation of cell proliferation and apoptosis (39). In the liver, hepatocyte growth factor–mediated hepatocyte proliferation involves the transport of HuR, an mRNA-binding protein that increases the half-life of certain cellular cyclins such as cyclin A2, D1, and D2, from the nucleus to the cytosol via phosphorylation and activation of AMPK (64). Interestingly, hepatocyte growth factor–induced AMPK activation and HuR translocation is strongly inhibited by SAMe through a process that involves the activation of PP2A (probably by methylation) and its association to AMPK (64). In the liver, after partial hepatectomy or liver injury, SAMe content drastically

decreases in response to MATI/III inactivation caused by stress (66), releasing the blockage this molecule exerts on AMPK activation and hepatocyte proliferation (35, 64) to facilitate liver regeneration and the recovery of normal liver mass and function. This effect of SAMe on AMPK activity is of particular interest because it links SAMe, for the first time, with glucose and fatty acid metabolism, which may be of crucial importance in explaining the development of fatty liver in humans and mice models defective in one-carbon metabolism, as well as in providing a mechanism to explain the inhibitory effect of SAMe on hepatocyte proliferation.

Role of Homocysteine

Although a variety of epidemiological studies have associated mild hyperhomocysteinemia with a variety of health conditions, including cardiovascular disease, end-stage renal disease, neural tube defects, and liver disease, the molecular mechanisms behind it are unclear (14, 67, 91). Hcy has been shown to inhibit the expression of a wide range of antioxidant enzymes and to induce endoplasmic reticulum (ER) stress in a variety of in vitro studies; moreover, hyperhomocysteinemia correlates with development of ER stress in an animal model of alcoholic liver injury (47, 48, 107, 115). ER stress is a condition in which unfolded and misfolded proteins accumulate, which then triggers the unfolded protein response, resulting in the activation of a number of transcription factors, including the sterol regulatory element-binding proteins (48). ER stress can lead to fatty liver disease, as sterol regulatory element-binding proteins induce genes involved in the cholesterol/triglyceride biosynthesis and uptake pathways (115). Finally, in vitro studies have shown Hcy to activate proinflammatory cytokines, possibly related to its ability to activate nuclear factor kappa B (48).

Hcy belongs to a group of molecules known as cellular thiols. The most abundant cellular thiols are GSH (the intracellular concentration of GSH ranges from 1 to 10 mmol/L) and cysteine (plasma total cysteine ranges from 200 to 300 μmol/L). The functions of these two thiols include maintaining intracellular redox homeostasis and facilitating the removal of toxic compounds; the thiols are also part of the cellular antioxidant defense system (67). Hcy is found at much lower concentrations than those of GSH and cysteine. The concentration of Hcy within the cell is around 1 μmol/L, and plasma ranges from 5 to 15 μmol/L, too low to play a major role in maintaining intracellular redox homeostasis. Hcy is in equilibrium with SAH (**Figure 1**), and although under normal conditions the removal of Hcy is sufficiently rapid to maintain the flux in the direction of hydrolysis, the synthesis of SAH is thermodynamically favored; consequently, conditions that impair Hcy removal (such as a deficiency in CBS and MTHFR) will lead to the accumulation of cellular SAH and to the inhibition of critical transmethylation reactions. It is therefore possible that SAH accumulation, altered SAMe/SAH ratio, and abnormal methylation reactions mediate the effects of hyperhomocysteinemia in the liver. Another reaction that may be involved in Hcy-mediated liver injury is the formation of Hcy thiolactone (**Figure 1**), a molecule that can react with lysine residues and free amine groups on numerous cellular proteins, leading to altered biological activity and premature degradation (45). Thiolactone formation, which is produced by an intramolecular condensation reaction between the thiol and the carboxylic acid of Hcy, is unique to this amino acid (cysteine has only a single carbon atom within the side chain whereas Hcy has two).

SUMMARY

A balance between the folate cycle, the methionine cycle, and the transsulfuration pathway (**Figure 1**) must be reached so that the cellular level of key metabolites such as SAMe, dTMP, and GSH and the SAMe/SAH ratio are not compromised, since this could lead to abnormal DNA synthesis (if the availability of dTMP is reduced), abnormal methylation reactions (if the cellular content of SAMe

and/or the ratio SAMe/SAH are reduced), or oxidative stress (if the content of GSH diminishes). Most, if not all, of the main genes involved in folate and methionine metabolism, including *GNMT*, *PEMT*, *CBS*, *MTHFR*, *TYMS*, and *CBS*, have genetic variants that may affect the activity or the expression of the enzymes encoded by these genes. The finding that a specific combination of *TYMS* and *MTHFR* polymorphisms associates with a reduced risk to develop HCC (118), together with the observation that certain *GNMT* (105) and *PEMT* (97) variants associate with increased risk to develop HCC and steatohepatitis, respectively, supports the concept that specific sets of folate- and methionine-metabolism gene polymorphisms may prevent or increase the risk of developing liver disease including fatty liver, cirrhosis, and HCC. The possibility of counteracting these genetic-associated alterations of folate and methionine metabolism, which are associated with an increased risk of developing liver injury, by the appropriate administration of specific nutrients (such as folate, methyl-THF, betaine, methionine, SAMe, or MTA) should be carefully examined as an important step toward the development of personalized medicine for the prevention of liver disease.

SUMMARY POINTS

1. SAMe is a methyl balance regulator: When the availability of methionine is low, hepatic SAMe content diminishes, releasing the inhibition this molecule exerts on the synthesis of methionine; conversely, when the concentration of methionine is elevated, hepatic SAMe content increases, causing the activation of methionine catabolism.
2. The main features of hepatic one-carbon metabolism are: (*a*) redundancy of the pathways that regenerate methionine and metabolize homocysteine; (*b*) a tight control by SAMe of the folate and methionine cycle as well as of the transsulfuration pathway; (*c*) a strong feedback inhibition by SAH of transmethylation reactions; and (*d*) a saving effect of methyl-THF on SAMe consumption.
3. Disruption of hepatic methyl balance in mice and humans often leads to changes in the concentration of SAMe, SAH, or the SAMe/SAH ratio, which may affect transmethylation reactions, and to the development of liver injury, mainly fatty liver disease and hepatocellular carcinoma.
4. Disorganization of the normal pattern of DNA and histone methylation may be central to liver disease induced by disruption of one-carbon metabolism.
5. Loss of expression of the liver-specific methionine metabolism genes *MAT1A*, *GNMT*, and *PEMT* is associated with the development of hepatocellular carcinoma in humans.
6. Through its inhibition of AMPK activity, a key regulator of cellular energy balance and cell proliferation, SAMe connects the methionine cycle with lipid and carbohydrate metabolism and provides a mechanism to explain the development of fatty liver and hepatocellular carcinoma induced by disruption of one-carbon metabolism.
7. A balance between the folate cycle, the methionine cycle, and the transsulfuration pathway must be reached so that the cellular level of key metabolites, such as dTMP, SAMe, and glutathione, and the SAMe/SAH ratio are not compromised, since this could lead to abnormal DNA synthesis, aberrant methylation reactions, or oxidative stress.

8. Genetic polymorphisms of some of the main enzymes involved in one-carbon metabolism have been identified that confer resistance or an increased risk of developing liver disease. This raises the possibility of correcting these genetic-mediated alterations of one-carbon metabolism by the personalized administration of specific nutrients, such as folate, methyl-THF, betaine, SAMe, or MTA.

FUTURE ISSUES

1. Several young individuals with *MAT1A* mutations leading to severe impairment of MATI/III activity have developed neurological disorders including brain demyelination. These findings raise the issue of how deficient hepatic SAMe synthesis affects brain function.
2. Generation of liver-specific, inducible *SAHH*-, *MS*-, and *BHMT*-knockout mice will be of great value for a better understanding of the role of homocysteine in liver pathophysiology.
3. Mice models are available devoid of the key enzymes of methionine and folate metabolism. A collaborative effort should be made to obtain genomewide information about DNA methylation, histone modifications maps from liver samples of these various mice models, and quantitative information about the plasma and liver content of the main methionine and folate metabolites. Bioinformatic analysis of these data may facilitate the identification of biomarkers and therapeutic targets for the treatment of liver disease.
4. Association studies should be carried out to determine the polymorphisms of the main genes involved in methionine and folate metabolism and risk of steatosis, steatohepatitis, and hepatocellular carcinoma, and the possibility of correcting these genetic-associated alterations of folate and methionine metabolism by the personalized administration of specific nutrients (folate, methyl-THF, betaine, methionine, SAMe, MTA) should be analyzed.

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DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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118. Describes results of epidemiologic study that shows genetic variants that lead to low hepatic MTHFR activity and increased TYMS activity reduce the risk to develop HCC.

119. Demonstrates hepatic steatosis in *PEMT* knockout mice.

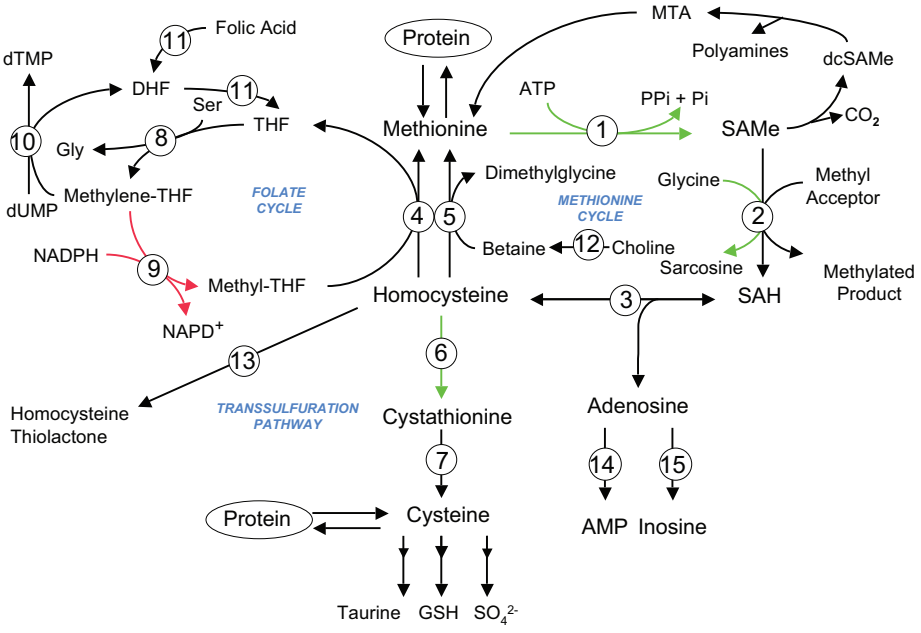

Figure 1

Hepatic methionine and folate metabolism. *S*-adenosylmethionine (SAMe) is generated from methionine and ATP in a reaction catalyzed by methionine adenosyltransferase (MAT, reaction *1*). SAMe is the principal biological methyl donor and a precursor for polyamine synthesis. Methylthioadenosine (MTA) is a by-product of polyamine synthesis and can be metabolized to regenerate methionine. In transmethylation, SAMe donates its methyl group to a large variety of acceptor molecules in reactions catalyzed by dozens of methyltransferase (reaction *2*), the most abundant in the liver being glycine *N*-methyltransferase (GNMT), that converts glycine into *N*-methyl-glycine (sarcosine). *S*-adenosylhomocysteine (SAH) is generated as a product of transmethylation and is hydrolyzed to form homocysteine and adenosine through a reversible reaction catalyzed by SAH hydrolase (SAHH, reaction *3*). Homocysteine can be remethylated to regenerate methionine by two enzymes: methyltetrahydrofolate-homocysteine methyltransferase (MS, reaction *4*, also called methionine synthase), and betaine-homocysteine methyltransferase (BHMT, reaction *5*). In the liver, but not in all tissues, homocysteine can also undergo the transsulfuration pathway to form cysteine via a two-step enzymatic process catalyzed by cystathionine β-synthase (CBS, reaction *6*) and cystathionase (reaction *7*). Cysteine is ultimately transformed into a variety of sulfur-containing molecules such as taurine, glutathione (GSH), and sulfate. Tetrahydrofolate (THF) is generated as a product of MS (reaction *4*) and is converted into 5,10-methylene-tetrahydrofolate (methylene-THF) by the enzyme methylene-THF synthase (reaction *8*) and then to 5-methyl-tetrahydrofolate (methyl-THF) by the enzyme methyl-THF reductase (MTHFR, reaction *9*). Alternatively, methylene-THF may be used for the synthesis of deoxythymidine monophosphate (dTMP) by the enzyme thymidine synthase (TYMS, reaction *10*). Dihydrofolate (DHF) is generated as a product of dTMP synthesis that can be converted to THF (reaction *11*). Choline oxidase (reaction *12*) converts choline to betaine (trimethylglycine), thiolactone synthase (reaction *13*) catalyzes the intramolecular condensation reaction between the thiol and the carboxylic acid of homocysteine, adenosine kinase (ADK, reaction *14*) catalyzes the synthesis of adenosine monophosphate (AMP), and adenosine deaminase (ADA, reaction *15*) converts adenosine into inosine. SAMe, SAH, and methyl-THF exert a tight metabolic control of hepatic methionine and folate metabolism. SAMe is an allosteric activator of MAT, GNMT, and CBS (in *green*) and an allosteric inhibitor of MTHFR (in *red*); SAH is a competitive inhibitor of many SAM-dependent methyltransferases, and methyl-THF is an allosteric inhibitor of GNMT. dcSAMe, decarboxylated SAMe.

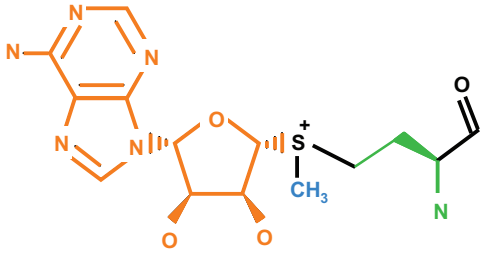

Figure 3

Structure of *S*-adenosylmethionine. SAMe participates in multiple cellular reactions. The methyl group of SAMe (in blue) is donated in transmethylation reactions, and the propylamino group (in green) is donated in polyamine synthesis. Radical SAMe enzymes use Fe_4S_4 clusters and SAMe to generate 5-deoxyadenosyl radicals (in orange). SAMe may bind to the CBS domain of a variety of proteins, such as the enzyme CBS, and to specific SAMe riboswitches. Metabolite RNA switches have been identified in all three kingdoms of life and typically regulate the expression of genes involved in the biosynthesis, transport, and utilization of the target metabolite. SAMe riboswitches have been identified in bacteria.

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Errata

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