Methionine metabolism and methyltransferases in the regulation of aging and lifespan extension across species

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Abstract
Methionine restriction (MetR) extends lifespan across different species and exerts beneficial effects on metabolic health and inflammatory responses. In contrast, certain cancer cells exhibit methionine auxotrophy that can be exploited for therapeutic treatment, as decreasing dietary methionine selectively suppresses tumor growth. Thus, MetR represents an intervention that can extend lifespan with a complementary effect of delaying tumor growth. Beyond its function in protein synthesis, methionine feeds into complex metabolic pathways including the methionine cycle, the transsulfuration pathway, and polyamine biosynthesis. Manipulation of each of these branches extends lifespan; however, the interplay between MetR and these branches during regulation of lifespan is not well understood. In addition, a potential mechanism linking the activity of methionine metabolism and lifespan is regulation of production of the methyl donor S-adenosylmethionine, which, after transferring its methyl group, is converted to S-adenosylhomocysteine. Methylation regulates a wide range of processes, including those thought to be responsible for lifespan extension by MetR. Although the exact mechanisms of lifespan extension by MetR or methionine metabolism reprogramming are unknown, it may act via reducing the rate of translation, modifying gene expression, inducing a hormetic response, modulating autophagy, or inducing mitochondrial function, antioxidant defense, or other metabolic processes. Here, we review the mechanisms of lifespan extension by MetR and different branches of methionine metabolism in different species and the potential for exploiting the regulation of methyltransferases to delay aging.

KEYWORDS
aging, lifespan, methionine restriction, methylation, methyltransferases, S-adenosylmethionine

1 | METHIONINE METABOLISM

Methionine is an essential proteogenic amino acid necessary for normal growth and development and is one of the four common sulfur-containing amino acids (methionine, cysteine, homocysteine, and taurine). In humans, methionine can be obtained from food or from gastrointestinal microbes. Methionine plays a well-known role as an initiator of protein synthesis in prokaryotes and eukaryotes and functions as an endogenous antioxidant at the surface of proteins (Levine, Mosoni, Berlett, & Stadtman, 1996; Luo & Levine, 2009).
In addition, methionine serves major roles through its metabolism, which fuels a variety of metabolic pathways. Methionine metabolism can be broken into three parts: the methionine cycle, the transsulfuration pathway, and the salvage cycle (Figure 1).

1.1 | Methionine cycle

The first step in methionine metabolism is performed by methionine adenosyltransferase (MAT), an enzyme conserved from Escherichia coli to humans that catalyzes the biosynthesis of S-adenosylmethionine (SAM) from methionine and ATP. SAM is the principal methyl donor and the second most widely used enzyme substrate after ATP (Cantoni, 1975). During substrate methylation, SAM donates its methyl group to acceptor molecules, for example, DNA, RNA, proteins, or other cellular metabolites, generating S-denosylhomocysteine (SAH). Over 200 known or putative methyltransferases have been identified in the human genome (Petrossian & Clarke, 2011) and 81 in yeast (Petrossian & Clarke, 2009). S-denosylhomocysteine hydrolase (SAHH/AHCY) catalyzes the reversible hydrolysis of SAH to adenosine and l-homocysteine. SAHH/AHCY proteins are tetramers with a NADH/NAD⁺ cofactor bound in the active site of each subunit (Brzezinski, Bujacz, & Jaskolski, 2008). There are also two AHCY-like proteins, AHCYL1 and AHCYL2, which most likely have lost their canonical enzymatic functions due to critical mutations in their AHCY domains. However, via hetero-multimerization, ACHYL1 and AHCYL2 can suppress the enzymatic activity of AHCY and thus act as dominant negative regulators of canonical AHCY (Devogelaere, Sammels, & Smedt, 2008). Cells must maintain low concentrations of SAH, which is a product inhibitor of SAM-dependent methylation reactions. Methyltransferases catalyze a variety of methylation reactions via the transfer of methyl groups on histone proteins as well as to nucleic acids, nonhistone proteins, and metabolites, although different methyltransferases exhibit different sensitivity to inhibition by SAH (Huang et al., 2000). Homocysteine can be remethylated to form methionine and retained in the methylation cycle, or converted to cysteine via the transsulfuration pathway and thus withdrawn from the methylation cycle. Remethylation of homocysteine to form methionine completes the methionine

![FIGURE 1 Schematic of methionine metabolism](image-url)
cycle. This process involves either methionine synthase (MS), which requires 5-methyltetrahydrofolate as a methyl donor, or betaine homocysteine methyltransferase (BHMT), which requires betaine as a methyl donor.

### 1.2 Transsulfuration pathway

Homocysteine from the methionine cycle can also be utilized in the transsulfuration pathway to produce cysteine. Cystathionine-δ-synthase is the first and rate-limiting enzyme of the transsulfuration pathway, the primary metabolic pathway for the synthesis of cysteine. Cystathionine-δ-synthase synthesizes cystathionine from the condensation of homocysteine and serine. Cystathionine is hydrolyzed by cystathionine-γ-lyase to produce cysteine, which is further used in the synthesis of proteins, glutathione, and taurine. Cystathionine-γ-lyase and cystathionine-δ-synthase also catalyze the production of hydrogen sulfide (H₂S) from cysteine and homocysteine. H₂S is a signaling molecule and cytoprotectant with a wide range of physiological functions. H₂S protects cells from oxidative stress and can modulate neuronal transmission, smooth muscle relaxation, release of insulin, and the inflammatory response. Activation of the transsulfuration pathway promotes the production of H₂S (Kabil, Vitvitsky, & Banerjee, 2014; Wallace & Wang, 2015).

### 1.3 Salvage pathway and polyamine biosynthesis

The methionine salvage pathway, or 5′-methylthioadenosine (MTA) cycle, regenerates methionine from SAM and is responsible for the production of polyamines (Minois, Carmona-Gutierrez, & Madeo, 2011; Pegg, 2016). In the methionine salvage pathway, SAM is decarboxylated by AdoMet decarboxylase into decarboxylated SAM (dcSAM), which serves as an aminopropyl group donor. In parallel, arginine converts arginine into ornithine, which is then decarboxylated by ornithine decarboxylase (ODC) to produce putrescine. Putrescine is further converted to spermidine and spermine through the consecutive action of two distinct aminopropyl transferases, spermidine synthase and spermine synthase, which use dcSAM as an aminopropyl donor. dcSAM is converted to MTA after the donation of an aminopropyl group for polyamine synthesis, and MTA is converted via six enzymatic steps back to methionine (Minois et al., 2011; Pegg, 2016). In addition to participating in methylation and synthesis of polyamines, SAM can also be activated by members of the radical SAM superfamily of enzymes that convert SAM to a highly oxidizing 5′-deoxyadenosyl radical intermediate involved in a variety of reactions (Landgraf, McCarthy, & Booker, 2016).

Beside its proteogenic and metabolic roles, methionine can also serve as an antioxidant. Methionine is one of the major targets of reactive oxygen species (ROS). Surface-exposed methionine residues of native proteins can be oxidized by ROS to R- and S-methionine sulfoxide, which can be reduced back to methionine by methionine sulfoxide reductases.

Consistent with the importance of methionine metabolism in cellular physiology, dysregulation of methionine metabolism has been reported in multiple diseases. Moreover, methionine restriction (MetR) has been tested as a treatment for disease in a number of clinical trials. Six genetic conditions in humans are known to lead to methionine and homocysteine elevation, that is, hypermethioninemasias and hyperhomocysteinemasias, as a result of deficiencies of enzymes involved in methionine metabolism (MAT, CBS, GNMT, AHCY) or affecting the mitochondrial transporter citrin and fumarylacetoacetate hydrolase (FAH; Mudd, 2011). Hyperhomocysteinemia is a characteristic of aging and upregulated levels of homocysteine contribute to the pathogenesis of multiple age-associated diseases, including cardiovascular dysfunction, decline in renal and cognitive functions, bone fractures, and others (Ostrakhovitch & Tabibzadeh, 2019). However, under certain circumstances hyperhomocysteinemia can be observed without any pathological changes; this raises a question whether homocysteine contributes to pathogenesis or only serves as a biomarker. In addition, multiple cancer types are auxotrophic for methionine due to changes in methionine metabolism (Agrawal, Alpini, Stone, Frenkel, & Frankel, 2012; Cavuoto & Fenech, 2012), a feature further exploited in a variety of therapeutic approaches (clinicaltrials.gov). In addition, ¹⁴C-methionine is the most popular amino acid tracer for PET imaging of brain tumors (Glaudemans et al., 2013). Finally, MetR in rodents not only extends lifespan but also protects from visceral fat mass accumulation and from the negative effects of a high-fat diet (Ables, Perrone, Orentreich, & Orentreich, 2012; Malloy et al., 2006; Orentreich, Matias, DeFelice, & Zimmerman, 1993; Wanders et al., 2018). Based on these results, MetR has also been tested in clinical trials of obese adults with metabolic syndrome (Plaisance et al., 2011).

In the following sections, we discuss how the different branches of methionine metabolism—the methionine cycle, the transsulfuration pathway, and polyamine metabolism—regulate lifespan (Figure 2) and discuss a potential mechanism linking methionine flux and lifespan regulation. We also review mechanisms of lifespan extension by MetR (Figure 3 and Table 1) with a main focus on the relevance of methionine metabolism and methyltransferases (Table 2) to lifespan extension. It should be noted that the mechanisms underlying lifespan extension by MetR and reprogramming of methionine metabolism (e.g., via activation of methionine metabolism) may overlap but are not identical. For example, both MetR and increasing SAH clearance as a result of the activation of flux via methionine metabolism can extend lifespan by impacting SAM/SAH level. However, as we discuss in this review, the methyltransferases affected by these two interventions and responsible for their lifespan phenotypes may differ.

### 2 Methionine metabolism and lifespan extension in yeast

The budding yeast *Saccharomyces cerevisiae* serves as one of the main model organisms for studying evolutionarily conserved mechanisms of aging and age-related diseases. There are two aging models in
budding yeast: chronological aging (CLS) and replicative aging (RLS). CLS is defined as the length of time that a nondividing yeast cell survives. RLS is defined as the number of daughter cells produced by a mother cell prior to senescence (Longo, Shadel, Kaeberlein, & Kennedy, 2012).

### 2.1 Methionine cycle

Wu et al. tested the effects of amino acids on CLS extension in the *S. cerevisiae* wild-type BY4742 strain, which is auxotrophic for his/leu/lys. They found that changing the ratio of nonessential and essential amino acids caused great changes in CLS, whereas increase or decrease in individual amino acids in the culture media had little effect. The exceptions were methionine restriction and addition of glutamic acid, which resulted in CLS extension (Wu, Song, Liu, & Huang, 2013). Methionine restriction can also be achieved by genetically limiting methionine biosynthesis. Yeast can use inorganic sulfur to produce sulfur-containing amino acids. To genetically induce MetR, Ruckenstuhl et al. and Johnson et al. used methionine-auxotrophic strains (genetic MetR) with limited (Δmet15) or no (Δmet2) endogenous methionine biosynthesis. These strains displayed enhanced CLS when grown on complete synthetic medium (i.e., with 30 mg/L methionine). In addition, restricting methionine from the medium increased CLS of the mutant strains but not of the prototrophic strain (Johnson & Johnson, 2014; Ruckenstuhl et al., 2014). Lifespan extension is often accompanied and dependent on TOR inhibition and activation of autophagy (Lopez-Otin, Blasco, Partridge, Serrano, & Kroemer, 2013; Parkhitko, Favorova, & Henske, 2013). In yeast, MetR is accompanied by activation of autophagy flux, and deletion of autophagy essential genes or decrease in vacuolar acidity...
via disruption of v-ATPase activity (which is responsible for the maintaining lysosomal pH) abolished MetR-induced CLS extension. Moreover, MetR-induced CLS extension could not be further extended by TOR inhibition, suggesting that MetR and TOR inhibition function via at least partially overlapping mechanisms (Ruckenstuhl et al., 2014). In various organisms including yeast, nuclear–mitochondrial communication is a key player in aging. Induction of the retrograde response pathway, which transmits signals of mitochondrial

| TABLE 1 | Manipulations of methionine metabolism that affect lifespan |
|---------|-----------------------------------------------------------|
| Gene/metabolite | Manipulation | Description | Effect on lifespan | References |
| **Yeast (Saccharomyces cerevisiae)** | | | | |
| Methionine | Restriction | Methionine restriction from the culture media | CLS extension | Johnson and Johnson (2014), Ruckenstuhl et al. (2014), Wu et al. (2013) |
| met15 | Deletion | Limited endogenous methionine biosynthesis | CLS extension | Johnson and Johnson (2014), Ruckenstuhl et al. (2014) |
| met2 | Deletion | No endogenous methionine biosynthesis | CLS extension | Johnson and Johnson (2014), Ruckenstuhl et al. (2014) |
| met3 | Deletion | Sulfate assimilation and synthesis of methionine | RLS extension | McCormick et al. (2015) |
| sam1 | Deletion | Methionine adenosyltransferase | RLS extension | McCormick et al. (2015) |
| sam1 | Overexpression | Methionine adenosyltransferase | CLS extension | Ogawa et al. (2016) |
| sam2 | Overexpression | Methionine adenosyltransferase | CLS extension | Ogawa et al. (2016) |
| SAH | Supplementation | S-adenosylhomocysteine supplementation | CLS extension | Ogawa et al. (2016) |
| cys4 | Deletion | Cystathionine beta-synthase | CLS extension | Laschober et al. (2010) |
| NaHS | Supplementation | H₂S donor | CLS extension | Hine et al. (2015) |
| GYY4137 | Supplementation | H₂S donor | CLS extension | Hine et al. (2015) |
| Spermidine | Supplementation | Polyamine | CLS and RLS extension | Eisenberg et al. (2009) |
| **Worms (Caenorhabditis elegans)** | | | | |
| Sams-1 | Downregulation | Methionine adenosyltransferase | LS extension | Hansen et al. (2005) |
| Metformin | Supplementation | Metformin inhibits bacterial folate and methionine metabolism | LS extension | Cabreiro et al. (2013) |
| anmt-1 | Overexpression | Lowers level of SAM | LS extension | Schmeisser and Parker (2018) |
| cbs-1 | Overexpression | Cystathionine beta-synthase | LS extension | Hine et al. (2015) |
| NAC | Supplementation | N-acetyl-l-cysteine supplementation | LS extension | Oh et al. (2015) |
| Spermidine | Supplementation | Polyamine | LS extension | Eisenberg et al. (2009) |
| **Flies (Drosophila melanogaster)** | | | | |
| Methionine + Amino acids | Restriction | Methionine restriction with reduced levels of amino acids | LS extension | Lee et al. (2014) |
| dAhcyL1 | Downregulation | AHCY-like protein 1 | LS extension | Parkhitko et al. (2016) |
| dAhcyL2 | Downregulation | AHCY-like protein 2 | LS extension | Parkhitko et al. (2016) |
| Sams | Downregulation | Methionine adenosyltransferase | LS suppression | Obata and Miura (2015) |
| dCbs | Overexpression | Cystathionine beta-synthase | LS extension | Kabil et al. (2011) |
| Gclc | Overexpression | Glutamate–cysteine ligase, catalytic subunit | LS extension | Orr et al. (2005) |
| Gclm | Overexpression | Glutamate–cysteine ligase, modulatory subunit | LS extension | Orr et al. (2005) |
| NAC | Supplementation | N-acetyl-l-cysteine supplementation | LS extension | Brack et al. (1997) |
| Spermidine | Supplementation | Polyamine | LS extension | Eisenberg et al. (2009) |
| **Mammals** | | | | |
| Methionine | Restriction | Methionine restriction in rats, mice, and human cells | LS extension | Koziel et al. (2014), Miller et al. (2005), Orentreich et al. (1993) |
stress to the nucleus, can increase replicative lifespan in yeast (Kirchman, Kim, Lai, & Jazwinski, 1999). MetR-induced CLS extension is dependent on the key mediator of retrograde signaling, the transcription factor RTG3, and 20% of differentially expressed genes under genetic MetR are reversed to basal levels in the absence of RTG3 (Johnson & Johnson, 2014).

Interventions that extend lifespan often confer resistance to different stresses. In agreement with this, genetic MetR makes yeast cells more resistant to oxidative damage, heavy metal stresses (Singh & Sherman, 1974), heat stress, and lack of divalent metal cations, which is highly toxic to yeast cells (Johnson & Johnson, 2014). Unbiased genetic screening of 4,698 viable single-gene deletion strains for replicative lifespan in S. cerevisiae identified that deletion of MET3, which encodes an enzyme important for the sulfate assimilation and synthesis of methionine, or SAM1, which encodes methionine adenosyltransferase, significantly extend replicative lifespan.

### TABLE 2 Manipulations of methyltransferases that affect lifespan

| Gene/metabolite | Manipulation | Description | Effect on lifespan | References |
|-----------------|-------------|-------------|--------------------|------------|
| Yeast (Saccharomyces cerevisiae) | | | | |
| trm9 | Deletion | tRNA methyltransferase | CLS extension | Fabrizio et al. (2010) |
| rcm1/nsun5 | Deletion | rRNA cytosine methylase | CLS extension | Schosserer et al. (2015) |
| tgs1 | Deletion | m5G cap formation of TLC1, RNA moiety of telomerase | RLS suppression | Franke et al. (2008) |
| set2 | Deletion | H3K36-specific methyltransferase | RLS extension | Ryu et al. (2014) |
| set1 | Deletion | H3K4-specific methyltransferase | RLS suppression | Cruz et al. (2018), Ryu et al. (2014) |
| dot1 | Deletion | H3K79-specific methyltransferase | RLS suppression | Ryu et al. (2014) |
| Worms (Caenorhabditis elegans) | | | | |
| anmt-1 | Overexpression | Nicotinamide N-methyltransferase | LS extension | Schmeisser et al. (2013), Schmeisser and Parker (2018) |
| set-9 | Downregulation | SET-domain-containing methyltransferase | LS extension | Greer et al. (2010), Hamilton et al. (2005), Ni et al. (2012) |
| set-15 | Downregulation | SET-domain-containing methyltransferase | LS extension | Hamilton et al. (2005) |
| cec-3 | Downregulation | H3K9 methyltransferase | LS extension | Greer et al. (2010), Hamilton et al. (2005) |
| set-4 | Downregulation | H3K20 methyltransferase | LS extension | Greer et al. (2010) |
| set-2 | Downregulation | H3K4 methyltransferase | LS extension | Greer et al. (2010) |
| dot-1.1 | Downregulation | H3K79 methyltransferase | LS extension | Wilhelm et al. (2017) |
| mes-2 | Downregulation | P2G protein, H3K27 methylation | LS extension | Ni et al. (2012) |
| met-1 | Downregulation | H3K36 methyltransferase | LS decrease | Pu et al. (2015) |
| set-18 | Downregulation | H3K36 methyltransferase | LS extension | Su et al. (2018) |
| prmt-1 | Overexpression | asymmetric arginine methyltransferase | LS extension | Takahashi et al. (2011) |
| nsun-5 | Downregulation | rRNA methyltransferase | LS extension | Schosserer et al. (2015) |
| Flies (Drosophila melanogaster) | | | | |
| Gnm1 | Overexpression | Glycine N-methyltransferase | LS extension | Obata and Miura (2015) |
| PRC1 | Downregulation | H3K27 trimethyltransferase complex | LS extension | Ma et al. (2018) |
| PRC2 | Downregulation | H3K27 trimethyltransferase complex | LS extension | Ma et al. (2018), Siebold et al. (2010) |
| pcm1 | Overexpression | Protein carboxyl methyltransferase | LS extension | Chavous et al. (2001) |
| hen1 | Downregulation | 2′-O-methylation | LS suppression | Abe et al. (2014) |
| dNsun5 | Downregulation | rRNA methyltransferase | LS extension | Schosserer et al. (2015) |
| dDnmt2 | Overexpression | tRNA methyltransferase | LS extension | Lin et al. (2005) |
| Mammals | | | | |
| Pcm1 | Deletion | Protein carboxyl methyltransferase | LS suppression | Lowenson et al. (2001) |
| Dnmt3a | Deletion | de novo DNA methyltransferase 3a | LS suppression | Nguyen et al. (2007) |
| Nrm1 | Deletion | N-terminal methyltransferase | LS suppression | Bonsignore et al. (2015) |
in agreement with inhibition of endogenous methionine biosynthesis in Δmet15 and Δmet2 mutant strains. In contrast to previous findings, however, Ogawa et al. found that stimulation of SAM synthesis by overexpression of SAM1 or SAM2, or by external supplementation of SAH but not SAM, significantly extended CLS via Snf (AMPK) activation and sah-1 mutant had a shortened CLS (Ogawa et al., 2016).

2.2 | Histone methylation

Imbalance in SAM/SAH ratio alters histone methylation. Yeast cho2Δ mutant cells, that have increased SAM/SAH ratios, have striking increases of the tri-methylation H3K4me3, H3K36me3, and H3K79me3 marks (Ye, Sutter, Wang, Kuang, & Tu, 2017). The histone H3/H4 systematic mutant library is a versatile library of histone H3 and H4 mutants in which each residue has been substituted with alanine and alanine residues were substituted with serine. To test the importance of histone methylation to lifespan extension, Sen et al. (2015) screened this library and found that mutation of H3K36, a histone mark associated with transcriptional elongation and splicing, resulted in shorter replicative lifespan, whereas deletion of the only H3K36me3 demethylase, Rph1, extended lifespan. In contrast, Ryu et al. found that the replicative lifespan of set2Δ-deficient (H3K36-specific methyltransferase) cells was significantly extended, whereas the replicative lifespans of set1Δ-deficient (H3K4-specific methyltransferase) and dot1Δ (H3K79-specific methyltransferase)-deficient cells were significantly shortened (Ryu, Rhie, & Ahn, 2014). H3K4me3 is primarily deposited by the COMPASS complex. S. cerevisiae has a single COMPASS complex containing the catalytic SET-domain protein Set1 and core structural proteins Swd1, Swd3, Sdc1, Bre2, Swd2, and Spp1. Consistent with the previous report, Cruz et al. found that catalytic-inactive set1ΔH1017L, Δswd1, and Δspp1 mutant yeast strains exhibit significant RLS defect resulting specifically from loss of H3K4me3 (Cruz et al., 2018).

2.3 | Nonhistone methylation

In light of the importance of methionine metabolism as a donor of SAM, growth in methionine-deficient media causes tRNA hypomethylation (Fesneau, Robichon-Szulmajster, Fradin, & Feldmann, 1975). Johnson and Johnson (2014) showed that genetic MetR via tRNA accumulation inhibits cytochrome C function and activates the retrograde response, which is required for MetR-induced CLS extension. Genome-wide studies and hypothesis-driven approaches have identified several specific methyltransferases important for extension of yeast lifespan. A screen of 4,800 viable deletion mutants in S. cerevisiae (mentioned above) identified several processes including tRNA methylation that modulates yeast CLS. Deletion of TRM9, encoding a tRNA methyltransferase that methylates uridine residues at the wobble position in tRNA(Glu), tRNA(Gly), tRNA(Lys), tRNA(Gln), and tRNA(Arg3), almost tripled yeast mean CLS under starvation/extreme CR and increased heat resistance, but reduced resistance to acetic acid (Table 2). Modifications in the anticodon loops of tRNAs determine the rate of translation, as well as the accuracy and fidelity of translation. However, how deletion of TRM9 interacts with MetR is not known (Fabrizio et al., 2010). Interestingly, Laxman et al. showed that the level of tRNA uridine thiolation reflects sulfur-containing amino acid availability and connects translational capacity with amino acid homeostasis. Decreased availability of cysteine and methionine causes decreased tRNA uridine thiolation that suppresses translation and cell growth. Thiolation-deficient yeast strains (Δubq4 and Δurm1) and mcm5-deficient strains (Δelp3 and Δtrm9) have increased CLS compared to controls. Uriddine thiolation might represent the critical connection point between methionine metabolism activity, growth, and longevity (Laxman et al., 2013). Schosserer et al. found that RCM1/Nsm5 (rRNA cytosine methylase) was downregulated in chronologically aged yeast cells and CLS in rcm1Δ-knockout cells was significantly increased (although replicative lifespan was reduced). In addition, these rcm1Δ-knockout yeast cells were resistant to H2O2 (Schosserer et al., 2015).

Saccharomyces cerevisiae telomerase consists of three protein subunits, Est1, Est2, and Est3, and an RNA moiety, TLC1. TLC1 contains a methyl-2,2,7,7-tetramethylorotane (m7G or TMG) cap at its 5′ end. Franke et al. demonstrated that m7G cap formation of TLC1 depends on the methyltransferase Tgs1 and that this TLC1 modification influences telomere length and structure. Moreover, cells lacking Tgs1 activity exhibited premature aging (decreased replicative lifespan) (Franke, Gehlen, & Ehrenhofer-Murray, 2008). It would be interesting to test how MetR affects the activity of Tgs1 methyltransferase and telomere length.

2.4 | Transsulfuration pathway

The transsulfuration pathway allows interconversion of homocysteine and cysteine via the intermediary formation of cystathionine. S. cerevisiae possess two active transsulfuration pathways (Thomas & Surdin-Kerjan, 1997), but mammalian cells can only convert homocysteine to cysteine. Laschober et al. identified gene expression signatures of cellular aging that are conserved between different human tissues and tested potential candidate genes for CLS extension in yeast. They found that deletion of CYS4, which encodes cystathionine beta-synthase (CBS) or PDX3 (Pyridoxine [pyridoxamine] phosphate oxidase), significantly extended CLS (Laschober et al., 2010). Dietary restriction in yeast via reduction of glucose in growth media promotes lifespan and increases H2S production via the transsulfuration pathway. In agreement with the beneficial role of H2S, the addition of exogenous H2S donors NaHS and GYY4137 significantly increased CLS (Hine et al., 2015).

2.5 | Polyamine metabolism

Methionine metabolism is critical for the production of polyamines because it supplies an aminopropyl group necessary to the process (Minois et al., 2011; Pegg, 2016). Levels of the naturally occurring
polyamine spermidine decrease with age. Eisenberg et al. showed that exogenous supplementation of spermidine led to a stable increase in intracellular spermidine levels in aging yeast cells and furthermore increased chronological and replicative yeast lifespans. Similar to genetic MetR, this lifespan extension was dependent on induction of autophagy, as deletion of ATG7 (autophagy essential gene) compromised the lifespan-extending effects of spermidine supplementation. Moreover, Eisenberg et al. (2009) identified that polyamine supplementation required suppression of histone acetylation and the double mutant for IKI3, which encodes an essential subunit of the histone acetylating elongator complex, and the histone acetyltransferase SAS3, increased the lifespan of yeast cells and abrogated the lifespan-extending effects of spermidine. Similarly, McCormick et al. (2015) found in the above-mentioned screen that deletion of SAS3 significantly extends the yeast replicative lifespan.

Nutritional and genetic studies in yeast have identified roles for different branches of methionine metabolism in lifespan regulation, as well as identified specific relevant methyltransferases. Nevertheless, little is known about the crosstalk among various methionine-relevant processes and how the crosstalk might impact lifespan.

3 | METHIONINE METABOLISM AND LIFESPAN EXTENSION IN CAENORHABDITIS ELEGANS

3.1 | Methionine cycle

In an unbiased RNAi screen, Hansen et al. identified 23 new longevity genes in C. elegans that when knocked down, extend lifespan from 10% to 90% (Hansen, Hsu, Dillin, & Kenyon, 2005). One of these genes, sams-1/C49F5.1 encodes methionine adenosyltransferase, which catalyzes the biosynthesis of SAM, the first step in methionine metabolism. In C. elegans, the eat-2 mutant serves as a genetic model for studying DR, as mutation of eat-2 defects disrupts pharyngeal pumping and thus limits food intake. Hansen et al. found that knockdown of sams-1 extended lifespan in a daf-16-independent manner but failed to extend the lifespan of eat-2(ad1116) mutants. Knockdown of sams-1 did not affect pharyngeal pumping but similar to DR, knockdown of sams-1 resulted in slender worms with reduced brood size and delayed reproduction. Moreover, the level of sams-1 mRNA is reduced threefold in eat-2 mutants (Hansen et al., 2005). Ching, Paal, Mehta, Zhong, and Hsu (2010) showed that overexpression of sams-1 partially suppresses lifespan extension of DR worms and RNAi knockdown of sams-1 reduces the global translation rate. Lifespan extension via reduced activity of the first enzyme in the methionine cycle suggests that limiting flux through the pathway is beneficial for lifespan, preventing accumulation of harmful metabolites, and is consistent with the beneficial effects observed for MetR and DR. Consistent with this, metformin, a drug widely prescribed to treat type 2 diabetes, increases lifespan in C. elegans co-cultured with E. coli as a food source after treatment with metformin. Metformin inhibits folate production and methionine metabolism in the bacteria, leading to changes in methionine metabolism and a decrease in the level of SAM in the worms (Cabreiro et al., 2013).

Sirtuins are a family of histone deacetylases that require NAD⁺ as a cosubstrate. During sirtuin-mediated deacetylation of ε-lysine residues, NAD⁺ is converted into nicotinamide (NAM), which can be methylated by nicotinamide N-methyltransferase (encoded by amnt-1 in C. elegans) to 1-methylnicotinamide (MNA). Schmeisser et al. (2013) showed that ANMT-1 overexpression extends C. elegans lifespan. Later, Schmeisser et al. showed that ANMT-1 competes for SAM with the methyltransferase leucine carboxyl methyltransferase 1 (LCMT-1), which is known to regulate autophagy. High NNMT activity leads to low SAM levels (similar to DR), serving as a starvation signal and thereby inducing autophagy. In addition, expression of ANMT-1 only in dopaminergic neurons suppressed age-dependent neurodegeneration and extended lifespan and was dependent on neuronal autophagy (Schmeisser & Parker, 2018). Moreover, Liu et al. (2019) showed that supplementation with glycine significantly prolongs C. elegans lifespan via feeding into the methionine cycle and that mutations in components of this cycle, methionine synthase (metr-1) and S-adenosylmethionine synthetase (sams-1), completely abrogate glycine-induced lifespan extension.

3.2 | Histone methylation

In a large-scale RNAi screen, Hamilton et al. identified 89 new genes that extend lifespan in C. elegans. Three of these genes, F15E6.1/Thr-9, R11E3.4/Set-15, and T09A5.8/cec-3, are predicted to encode proteins that contain a SET domain, a signature motif of most histone methyltransferases (Hamilton et al., 2005).

3.3 | H3K4 (activation histone mark)

The methylation state of H3K4 strongly correlates with transcriptional activation, and methylation status at H3K4 depends on the activity of histone lysine methyltransferases (KMTs), histone lysine demethylases (KDMs), and can be affected by the activity of methionine metabolism. C. elegans with reduced function of sams-1 and concomitant decrease in SAM have a broad decrease in the global H3K4me3 levels (Ding et al., 2015). KDM1 (in worms, KDM1A—spr-5 and Isd-1, KDM1B—amx-1) removes only Me1 and Me2 from H3K4, and KDM5 (bdr-2 is a sole KDM5 member in C. elegans) can remove Me3 and Me2 from H3K4. Greer et al. performed a targeted RNAi screen by selecting genes that encode known worm methyltransferases and identified that knockdown of set-4, set-9, set-15, and members of ASH-2 trithorax complex (ASH-2, WDR-5, and SET-2), which trimethylates histone H3 at lysine 4 (H3K4), extended C. elegans lifespan. Further, overexpression of RBR-2 (H3K4me3 demethylase that opposes the activity of ASH-2 trithorax complex by removing methyl marks) extends lifespan (Greer et al., 2010). Strikingly, the effect of ASH-2, WDR-5, or SET-2 depletion on lifespan is inherited through several generations (wild-type F3 and F4 descendents from ash-2, wdr-5, or set-2 mutants were long-lived).
(Greer et al., 2011). Surprisingly, deletion of H3K4me2 demethylase, spr-5, also causes a trans-generational increase in lifespan. The lifespan extension induced by disruption of the H3K4 trimethylase complex occurs immediately after deletion of H3K4 trimethylase complex and disappears after three generations. Different from this, deletion of spr-5 initially has no observable effect but a longevity extension appears after seven generations (Greer, Becker, Latza, Antebi, & Shi, 2016). McColl et al. (2008) found that exposure to lithium throughout adulthood increased longevity via a sharp reduction of expression of H3K4 histone demethylase LSD1 (KDM1A) and that knockdown of LSD1 is sufficient to extend lifespan. In agreement with these observations, Alvesares et al. found that a rbr-2 mutant strain has a longer lifespan, and both rbr-2 and spr-5 are required for extended longevity in daf-2 adults, suggesting their anti-aging function (Alvares, Mayberry, Joyner, Lakowski, & Ahmed, 2014). Further, Wilhelm et al. performed a screen for genes that extend postreproductive lifespan using an RNAi library targeting 800 factors involved in chromatin and transcriptional regulation. Among the 36 longevity genes identified in the screen were rbr-2 (H3K4me3 demethylase) and dot-1.1 (H3K79-specific methyltransferase) (Wilhelm et al., 2017). However, there is conflicting evidence with regard to the effects of up- or downregulation of H3K4 methylation on lifespan extension, as downregulation of either H3K4 methyltransferase complex or demethylase can prolong lifespan. H3K4 methylation might be a potential mechanistic target for MetR, as this is among the most sensitive histone methylation site that is suppressed under MetR (Mentch et al., 2015).

3.4 | H3K27 (repressive histone mark)

Jin et al. observed increased activity of the H3K27 demethylase UTX-1 in C. elegans during aging and corresponding loss of H3K27me3 on the regions corresponding insulin/IGF-1 signaling pathway components. RNAi knockdown of UTX-1, which extends the lifespan of C. elegans by 30%, does not have an additive effect when combined with mutation of daf-2, and increases H3K27me3 on the lgl1/daf-2 gene (Jin et al., 2011). In a targeted RNAi screen focused on histone demethylases, Maures et al. identified four histone demethylases that regulate C. elegans lifespan, UTX-1 (KDM6A, H3K27me2/me3 demethylases), RBR-2 (JARID1/KDM5B, H3K4me2/me3 demethylases), LSD-1 (KDM1A; H3K4me1/me2, H3K9me2 demethylases), and T26A5.5 (JHDM1B/KDM2B, H3K36me2 demethylases). Moreover, Ni, Ebata, Alipanahiramadi, and Lee (2012) performed a small-scale RNAi screen targeting most putative histone methyltransferases and demethylases in worms and identified six longevity genes that, when inactivated by RNAi, can extend worm lifespan: set-9/26 (MLLS), mes-2 (E(ZI)), utx-1 (UTX), jmjd-2 (KDM4), and rbr-2 (JARID1). Four of these six genes, set-9, set-26 (H3K9 trimethyltransferase), rbr-2, and utx-1, were previously identified as longevity-associated genes in worms. In contrast to lifespan extension by UTX-1 downregulation, overexpression of another H3K27me3 demethylase, JMJD-3.1, extended lifespan (Labbadia & Morimoto, 2015). Overexpression of the H3K9 and H3K27me2/3 demethylase jmjd-1.2, but not of the catalytically inactive jmjd-1.2<sup>cat</sup>, led to significantly increased longevity, whereas downregulation of jmjd-1.2 and jmjd-3.1 suppressed ETC-mediated longevity in C. elegans (Merkwirth et al., 2016).

3.5 | H3K36 (histone mark, associated with transcriptional elongation and splicing)

Deletion of met-1 H3K36 methyltransferase decreased lifespan in C. elegans and increased the level of mRNA expression noise with age (Pu et al., 2015). Su et al. compared expression of known histone methyltransferases and proteins containing the SET enzymatic domain of methyltransferases in young and old C. elegans and found that the levels of set-2, set-10, set-18, set-32, and F54F7.7 were up-regulated in old worms. They further demonstrated that like downregulation of the known lifespan regulator set-2, downregulation of set-18 also led to extended lifespan, and identified set-18 as a histone H3K36 dimethyltransferase. They demonstrated that SET-18 represses daf-16a promoter through H3K36me2 modification and muscle-specific expression of SET-18 in old worms was responsible for shortening lifespan (Su et al., 2018).

3.6 | Nonhistone methylation

Takahashi et al. (2011) studied arginine methylation, a posttranslational protein modification, and identified PRMT-1 as the major asymmetric arginine methyltransferase in C. elegans. They demonstrated that PRMT1 methylates DAF-16, thereby blocking its phosphorylation by AKT. In addition, loss of prmt-1 shortened lifespan and overexpression of PRMT1 significantly increased lifespan when co-overexpressed with DAF-16.

In yeast, rcm1-knockout cells have an increased replicative lifespan. In C. elegans, depletion of RCM1/nsun-5 (255 rRNA cytosine 2278-specific methylase) by RNAi extended the lifespan of worms with complete removal of food during adulthood or in eat-2 mutant worms, but the effect was completely abrogated in worms fed ad libitum (Schosserer et al., 2015).

3.7 | Transsulfuration pathway

As noted above, in C. elegans, the eat-2 mutant strain serves as a genetic model for studying DR. Eat-2 mutant worms produced more H<sub>2</sub>S, a product of the transsulfuration pathway, than wild-type worms. CBS-1 is a worm ortholog of cystathionine-β-synthase, the rate-limiting enzyme in the transsulfuration pathway (Figure 1). RNAi knockdown of cbs-1 decreased the lifespan extension normally associated with eat-2 mutants, and overexpression of CBS-1 in wild-type worms prolonged lifespan (Hine et al., 2015). Supplementation of the diet with the product of the transsulfuration pathway, N-acetyl-l-cysteine, significantly extended both the mean and maximum lifespan and significantly increased resistance to oxidative stress, heat stress, and UV irradiation in C. elegans (Oh, Park, & Park, 2015).
3.8 | Polyamine metabolism

Similar to yeast, supplementation of the food with spermidine in C. elegans induced autophagy and prolonged lifespan by up to 15%, whereas knockdown of Bcl-1, a gene essential for autophagy, abolished the spermidine-mediated increase in lifespan (Eisenberg et al., 2009).

4 | METHIONINE METABOLISM AND LIFESPAN EXTENSION IN DROSOPHILA MELANOOGASTER

4.1 | Methionine cycle

DR is one of the most effective dietary interventions that extends lifespan in diverse organisms (Tatar, Post, & Yu, 2014), but also leads to reduced fecundity. Methionine supplementation alone can increase fecundity in DR animals to levels comparable to full-fed controls without reducing lifespan (Grandison, Piper, & Partridge, 2009). Several groups have developed semi- or fully defined fly diets for testing the effects of specific food components on lifespan. The basal level of methionine significantly varies in these diets, ranging from 0.34 to 1.65 g/L (Piper, 2017). To test the effect of MetR in flies and its interaction with DR, Lee et al. compared lifespans of flies using diets with different levels of amino acids and different concentrations of methionine. Restriction of amino acids increased lifespan at any concentration of methionine, whereas MetR extended lifespan only in conditions when the levels of amino acids were reduced (Lee et al., 2014). Interestingly, we recently showed that naturally selected long-lived flies, which have twice the lifespan of wild-type strains, have higher levels of endogenous methionine, suggesting that high levels of methionine are not detrimental to lifespan and that flux via methionine metabolism is more critical than the level of methionine itself (Parkhitko et al., 2016). In agreement with this hypothesis, we suggest that methionine metabolism is reprogrammed during aging that leads to the accumulation of SAH and homocysteine. Downregulation of dAhcyL1/dAhcyL2 at the whole-organism and tissue-specific level extends lifespan and healthspan. In our model, downregulation of dAhcyL1/dAhcyL2 activates Ahcy13, which in turn promotes SAH and homocysteine processing, resulting in an increase in methionine flux and an effect reminiscent of methionine restriction (Parkhitko et al., 2016).

In agreement with the importance of flux via methionine metabolism, Obata et al. showed that overexpression of GNMT, which converts glycine to sarcosine (N-methyl-glycine) by methyl group transfer using SAM and functions as a regulator of SAM levels in metabolic organs, suppresses age-dependent SAM increase and extends lifespan (Obata & Miura, 2015). Interestingly, this group also found that, in contrast to C. elegans where sams-1/C49F5.1 (methionine adenosyltransferase) catalyzes the biosynthesis of SAM, the first step in methionine metabolism) extended lifespan, knockdown of Sams in flies significantly shortened lifespan. Notably, there are four Sams genes in C. elegans but only one in Drosophila, such that suppression of Sams in Drosophila, but not in C. elegans, should not block the methionine cycle. Interestingly, tissue-specific activation of methionine metabolism flux via downregulation of dAhcyL1/L2 in the brain or intestine extends healthspan and lifespan (Parkhitko et al., 2016). In accordance, Obata et al. (2018) identified SAM as a critical regulator of stem cell division in the Drosophila midgut, acting via regulation of Dph5, HemK1, and HemK2 methyltransferases and control of protein translation. Based on this, we suggest that tissue-specific dysregulation of methionine metabolism can be a driving force of age-dependent intestinal dysplasia and further, that either MetR or activation of methionine flux can prevent these changes, improve proliferative homeostasis, and extend lifespan. Furthermore, Kashio et al. (2016) found that alterations of SAM metabolism in the fat body via up- or downregulation of GNMT affect repair of wing disks, showing that organ-specific changes in methionine metabolism have systemic effects on regenerative processes. It will be of interest to further understand how methionine metabolism is altered with age in different organs and create genetic tools that will allow dissection of the role(s) of methionine metabolism in specific tissues and organs.

Several histone and nonhistone methyltransferases in flies have been shown to regulate lifespan, as discussed below.

4.2 | Histone methylation

4.2.1 | H3K4 (activation histone mark)

Liu et al. tested the effects on histone methylation of three core enzymes involved in methionine metabolism, Sam-S, Ahcy13, and Cbs. They found that downregulation of Sams led to decreased levels of H3K4me3 and H3K9me2 in Drosophila S2 cells (Liu, Barnes, & Pile, 2015). Consistent with this, we found that activation of flux in methionine metabolism via downregulation of dAhcyL1 significantly suppressed the level of H3K4me3 (Parkhitko et al., 2016). The corepressor SIN3, which controls histone acetylation through association with the histone deacetylase RPD3, binds to the promoter regions of genes involved in methionine metabolism and regulates levels of SAM and H3K4me3 (Liu & Pile, 2017). Interestingly, Liu and Pile (2017) showed that SIN3 regulates crosstalk between histone acetylation and histone methylation via regulation of methionine metabolism.

4.2.2 | H3K27 (repressive histone mark)

The levels of H3K27 methylation have been shown to affect lifespan in Drosophila. Flies heterozygous for mutations in E(z) and esc, members of the PRC2 H3K27 trimethyltransferase complex, decreased levels of H3K27me3 and extended lifespan (Siebold et al., 2010). Ma et al. further confirmed this by demonstrating that levels of H3K27me3 increase with age in Drosophila and reduction of components of PRC2 (esc, E(z), Pcl, Su(z)2), and PRC1 (Psc and Su(z)2) promote lifespan. Moreover, they showed that PRCs-deficiency promotes glycolysis and increase in glycolytic
genes in wild-type animals extends longevity (Ma et al., 2018). As mentioned above, suppression of H3K27me3 demethylase (UTX-1) in worms also extends lifespan (different from what is observed for flies); however, overexpression of H3K27me3 demethylase JMJD-3.1 extended lifespan, suggesting that the genes regulated by H3K27me3 are organism- and tissue-specific. Also in contrast to C. elegans, in which suppression of RBR-2 results in elevated lifespan (Greer et al., 2010, 2011), Li, Greer, Eisenman, and Secombe (2010) showed that male flies mutant for little imaginal disk (lid), which encodes the Drosophila H3K4me3 demethylase, has a significantly shorter lifespan, and an effect that was not observed in females.

4.2.3 | H3K9 (repressive histone mark)

Whereas euchromatic regions (transcriptionally active) are characterized by increased levels of H3K4me3 and H3K36me3, and heterochromatic regions (transcriptionally silent) are characterized by having increased levels of H3K9me2 or 3, which are able to recruit heterochromatin protein-1 (HP-1) to advance heterochromatin formation (H3K9me2 can also mark inactive euchromatin). Aging is associated with loss of repressive heterochromatin marks and can be measured in Drosophila using a position effect variegated (PEV) LacZ reporter gene (Jiang et al., 2013), in which LacZ is inserted at the boundary between euchromatin and heterochromatin; the level of heterochromatization determines the level of LacZ expression. In Drosophila, specific heterochromatin regions are remodeled during aging and that lifespan-extending interventions such as calorie restriction suppress age-dependent heterochromatin remodeling (Jiang et al., 2013; Wood et al., 2010). Moreover, heterochromatin formation prolongs lifespan and heterochromatin formation depends on the levels of HP1 and H3K9 methylation (Larson et al., 2012). A significant decrease in both H3K4me3 and H3K36me3 was observed in older flies (Wood et al., 2010).

4.3 | Nonhistone methylation

Accumulation of damaged (isoaspartyl-containing) proteins has been demonstrated in different species including mice. The accumulation of these potentially dysfunctional isoaspartyl proteins is counteracted by a protein carboxyl methyltransferase (PCMT). Chavous et al. demonstrated that flies ubiquitously expressing PCMT have an increased lifespan at 29°C; however, lifespan extension was not observed at 25°C (Chavous, Jackson, & O’Connor, 2001). Abe et al. studied how microRNAs expression pattern changes with age. In Drosophila, most miRNAs are loaded into Ago1 and remain unmodified; however, some miRNAs are found to be 2′-O-methylated and loss of 2′-O-methylation of small RNAs leads to destabilization as well as tailing and trimming of small RNAs. In Drosophila, 2′-O-methylation of small RNAs depends on the methyltransferase Hen1 and is associated with loading of miRNAs into Ago2. Abe et al. found that age-associated increase in specific Drosophila miRNAs isoforms reflected increased 2′-O-methylation and loading of these isoforms into Ago2. They also found that the lack of 29-O-methylation by Hen1 and Ago2 mutations resulted in reduced lifespan and brain degeneration (Abe et al., 2014). Similar to yeast and worms, ubiquitous downregulation of RCM1/dNsun5 in male flies extended the lifespan of flies by 16%–20%, but the effect was abrogated on the diet that was richer in sugar and yeast. Overexpression of dNsun5 reduced mean lifespan by 58% (Schosserer et al., 2015). Lin, Tang, Reddy, and Shen (2005) demonstrated that overexpression of dDnmt2, a methyltransferase, can extend the Drosophila lifespan. Drosophila is thought to have no or low levels of genomic 5-methylcytosine. The human homolog of dDnmt2, TRDMT1/DNMT2, does not display DNA methyltransferase activity but can methylate aspartic acid tRNA (Goll et al., 2006).

4.4 | Transsulfuration pathway

Cystathionine β-synthase (CBS) catalyzes the first and rate-limiting step in the transsulfuration pathway, which involves pyridoxal 5′-phosphate-dependent condensation of serine and homocysteine to form cystathionine. Kabil et al. demonstrated that activity of dCBS and the transsulfuration pathway is increased under DR and inhibition of the second enzyme in transsulfuration pathway, γ-cystathionase, using propargylglycine, causes robust suppression of lifespan extension by DR but not in fully fed flies. In agreement with this, either ubiquitous adult-specific overexpression of dCBS or neuronal overexpression of dCBS is sufficient to increase longevity (Kabil, Kabil, Banerjee, Harshman, & Pletcher, 2011). In agreement with the positive role of the TSP in lifespan extension, maximal H2S production of flies subjected to various forms of Met and DR correlated with maximal lifespan extension (Hine et al., 2015). Cysteine is a product of the transsulfuration pathway and a precursor for the synthesis of tripeptide γ-glutamylcysteineylglycine (glutathione, GSH). GSH helps maintain cellular redox homeostasis, acts as a xenobiotic conjugant, facilitating export of xenobiotics from cells, participates in thiolation and dethiolation of proteins, and has additional roles. Glutamate-cysteine ligase (GCL) is the rate-limiting enzyme that conjugates glutamate and cysteine to create γ-glutamylcysteine. GSH synthase (GS) links glycine to γ-glutamylcysteine to form GSH. Glutamate-cysteine ligase (GCL) is a heterodimeric enzyme consisting of a catalytic subunit, GCLc, and a modulatory subunit, GCLm. Orr et al. (2005) showed that the overexpression of GCLc or GCLm in flies using either global or neuronal drivers of expression led to an increase in the glutathione content observed in fly homogenates and extended lifespan. In agreement with these findings, feeding flies with a cysteine donor for GSH, N-acetylcysteine (NAC), results in a dose-dependent increase in lifespan (Brack, Bechter-Thuring, & Labuhn, 1997).

4.5 | Polyamine metabolism

Similar to yeast and worms, supplementation in Drosophila of regular food with 1 mM spermidine was shown to prolong lifespan by up to 30% (Eisenberg et al., 2009).
5.1 Methionine cycle

It was first shown in rats that MetR leads to an increase in lifespan. The lifelong reduction of a single dietary component, methionine, from 0.86% to 0.17% in Fisher 344 rats resulted in 30% increase in male rat lifespan (Orentreich et al., 1993). In female CB6F1 mice, decrease in methionine from 0.43% by weight to 0.1%-0.15% increased lifespan, slowed immune and lens aging, and decreased levels of serum IGF-I, insulin, glucose, and thyroid hormone, despite an increase in food uptake (Miller et al., 2005). In mice, the ideal range of dietary methionine restriction is from 0.17% to 0.25% (Forney, Wanders, Stone, Pierce, & Gettys, 2017). Restriction of dietary methionine to levels above 0.25% was without effect while restriction to levels below 0.12% produced responses characteristic of essential amino acid deprivation. Although restriction of dietary methionine to 0.12% does not evoke essential amino acid deprivation responses, it provides insufficient methionine to support growth (Forney et al., 2017). In human diploid fibroblasts, reduction of methionine from 30 to 1 mg/L had no significant effect on the rate of cell proliferation in early passage cells but significantly extended their replicative lifespan, postponing cellular senescence. Extended lifespan was associated with reduced oxygen consumption (Koziel et al., 2014). In humans, an 83% reduction in daily methionine uptake for 3 weeks reduced plasma methionine levels and altered circulating metabolism with the most effect on cysteine and methionine metabolism (Gao et al., 2019). In addition, in humans, vegan diet is associated with decreased methionine content (McCarty, Barroso-Aranda, & Contreras, 2009). In mice, gene expression profiles of MetR and CR mice do not significantly overlap, suggesting that these dietary regimens affect longevity through partly independent pathways (Sun, Sadighi Akha, Miller, & Harper, 2009). MetR restores a younger metabolic phenotype in adult mice: MetR in 12-month-old mice (0.172% vs. 0.86% methionine) reversed age-induced alterations in body weight, adiposity, physical activity, and glucose tolerance to levels observed in healthy 2-month-old control-fed mice. MetR also causes lipid metabolism reprogramming in mice. Short-term (48 hr) MetR increases hepatic fibroblast growth factor-21 (FGF21) expression/secrection (Lees et al., 2014). In young animals, MetR stunts growth and development, reducing total length, levels of serum insulin-like growth factor 1 (IGF-1), and growth hormone signaling activity. FGF21 is an atypical FGF that is secreted by the liver during fasting and elicits diverse aspects of the adaptive starvation response. Multiple beneficial effects of FGF21 are similar to MetR. FGF21 induces hepatic fatty acid oxidation and ketogenesis, increases insulin sensitivity, blocks somatic growth, and causes bone loss. Transgenic mice overexpressing FGF21 are markedly smaller, have decreased circulating IGF-1 concentrations, and have a significantly extended lifespan (Zhang et al., 2012). The multiple physiological outcomes of MetR were recently reviewed and will not be further discussed here (Ables, Hens, & Nichenametla, 2016; Cuvuoto & Fenech, 2012; Lee, Kaya, & Gladyshev, 2016; McIsaac, Lewis, Gibney, & Buffenstein, 2016; Sanchez-Roman & Barja, 2013; Zhou et al., 2016). Methionine metabolism is also altered in the tissues of long-lived Ames mice (Uthus & Brown-Borg, 2006) and naked mole-rats (Ma et al., 2015); however, whether these changes are causative or correlative is not known. Interestingly, Gu et al. recently demonstrated that SAM disrupts the SAMTOR-GATOR1 complex by binding directly to SAMTOR. GATOR1, the GTPase activating protein for RagA/B, promotes the localization of mTORC1 to the lysosomal surface, its site of activation. MetR reduces SAM levels and promotes the association of SAMTOR with GATOR1, thereby inhibiting mTORC1 signaling in a SAMTOR-dependent fashion (Gu et al., 2017). Because mTORC1 is a central regulator of aging (Parkhitko, Favorova, Khabibullin, Anisimov, & Henske, 2014; Saxton & Sabatini, 2017), SAMTOR, a SAM sensor, might serve as a critical connection hub between methionine metabolism, mTORC1 signaling, and aging.

Another important player in the methionine cycle and aging is homocysteine. In human plasma, total homocysteine is present in four forms: 1%-2% as the thiol form, homocysteine; 82%-83% combined in disulfide linkage with cysteines of proteins (mostly albumin); and the remaining 15%-16% as the free disulfides homocysteine and cysteine–homocysteine disulfide (all these fractions are called total homocysteine, tHcy). One reason homocysteine can be harmful is because homocysteine can be converted to thiolactone as a result of an error-editing function of some aminoacyl-tRNA synthetases. Thiolactone is chemically reactive and acylates free amino groups in proteins. The amount of thiolactone formation is dependent on methionine flux (Jakubowski, Zhang, Bardeguez, & Aviv, 2000). A homocysteine thiolactonase, paraoxonase I, detoxifies thiolactone via hydrolysis. In addition, homocysteine can cause neuronal death serving as an agonist for metabotropic glutamate receptors as well as for NMDA and AMPA/kainite ionotropic glutamate receptors particularly via ERK activation (Poddar & Paul, 2009). Moreover, high levels of homocysteine upregulate mTORC1 activity. Homocysteine is sensed by protein complex composed of leucyl-tRNA-synthetase and folinic acid, which regulates activity of mTORC1 via its tethering to lysosomal membranes (Khayati et al., 2017). Chronic age-dependent inhibition of the flux via methionine metabolism would result in upregulated levels of homocysteine. One of the consequences of high level of homocysteine would be activation of mTORC1 and chronic inhibition of the molecular clearance of protein products. Interestingly, in both mice and rats MetR leads to hyperhomocysteinemia (Ables et al., 2015; Elshorbagy et al., 2010; Perrone et al., 2012; Tamanna, Mayengbam, House, & Treberg, 2018). Further studies are required to clarify why MetR extends lifespan and exerts multiple benefits while increasing the level of potentially harmful homocysteine.

5.2 DNA, histone, and nonhistone methylation

In general, aging is associated with DNA hypomethylation; however, some DNA regions become hypermethylated. In mammals, DNA methylation patterns change with age (Hannum et al., 2013)
and can serve as a marker for chronological age (Horvath, 2013), but it is not known whether these changes are causative or correlative. As mentioned above, methionine metabolism is significantly altered in the tissues of long-lived Ames mice (Uthus & Brown-Borg, 2006). Similar to the expression of methionine metabolism genes, DNMTs (DNA methyltransferases) are also differentially expressed in Ames mice compared with wild-type siblings, potentially creating a regulatory loop between methionine metabolism, DNA methylation and lifespan regulation (Armstrong, Rakoczky, Rojanathammanee, & Brown-Borg, 2014). Moreover, age-associated methylation changes are suppressed in longer-lived Ames dwarf, calorie-restricted and rapamycin-treated mice (Cole et al., 2017; Wang et al., 2017). Mattocks et al. (2017) demonstrated that MetR improves the efficiency of DNA methylation maintenance systems in adult mice but had no effect in young mice and the effect of MetR on DNA methylation was tissue-specific and was driven by changes in SAH. We hypothesize that the activity of methionine metabolism is directly sensed by DNA methyltransferases that set up the biological age reflected in epigenetics clocks. Based on this hypothesis, various lifespan-extending regimens would reset the epigenetic clocks via altering the activity of methionine metabolism that in turn would determine the activity if DNA methyltransferases. A regulatory loop between DNA methylation and methionine metabolism has been described by Zhou et al. They showed that H19 IncRNA binds to and inhibits S-adenosylhomocysteine hydrolase (SAHH) and that this interaction prevents SAHH from hydrolyzing SAH, leading to block of DNA methylation by DNMT3B at numerous genomic loci (Zhou et al., 2015). Mitochondrial dysfunction, which is commonly observed with aging, can also alter DNA methylation patterns via effects on methionine metabolism; namely, leading to an increase in the SAM/SAH ratio and an increase in DNA methylation (Lozoya et al., 2018).

In contrast to yeast, worms, and flies, for mice, information about specific methyltransferases capable of extending lifespan is limited. As discussed earlier, flies expressing PCMT have an extended lifespan (Chavous et al., 2001), whereas Pcmter/- mice die at a mean age of 42 days from seizures (Lowenson, Kim, Young, & Clarke, 2001). Depleting the H3K9me3 methyltransferase Suv39h1 in a progeria mouse model reduces H3K9me3 levels, delays senescence in progeroid cells, and extends lifespan, but whether perturbation of Suv39h1 can extend lifespan in healthy mice is not known (Liu et al., 2013). Mice lacking functional de novo DNA methyltransferase Dnmt3a are born healthy but degenerate in adulthood and die prematurely (Nguyen, Meletis, Fu, Jhaveri, & Jaenisch, 2007). Mice deficient for NRM1, N-terminal methyltransferase, which regulates protein-DNA interactions, exhibit phenotypes associated with impaired DNA repair and premature aging (Bonsignore et al., 2015).

### 5.3 Transsulfuration pathway

Similar to worms and flies, a heterogeneous stock of mice (NIA Interventions Testing Program) fed with N-acetyl-γ-cysteine (NAC) had a significantly extended lifespan. Notably, the effect was sex-specific. In females, NAC treatment did not significantly affect total lifespan. In males, both high (1,200 mg kg⁻¹ day⁻¹) and low (600 mg kg⁻¹ day⁻¹) NAC doses increased total lifespan. However, both doses of NAC caused a sudden drop in body weight, followed by a further slow decline (Flurkey, Astle, & Harrison, 2010). A characteristic of aging and a major cause of mortality and morbidity is the age-dependent decline in capillary density and blood flow. Recently, two groups showed that H₂S, a product of enzymes with roles in transsulfuration pathway, promotes angiogenesis (Das et al., 2018; Longchamp et al., 2018). Longchamp et al. demonstrated that MetR increased VEGF expression in vitro and increased capillary density in mouse skeletal muscle in vivo via the GCN2/ATF4 amino acid starvation response pathway. This effect was independent of hypoxia or HIF1α. Cystathionine-γ-lyase was required for VEGF-dependent angiogenesis in part by inhibiting mitochondrial electron transport chain activity (Longchamp et al., 2018). Das et al. demonstrated that increasing the expression of SIRT1 in endothelial cells is sufficient to increase the capillary density of skeletal muscle, enhance exercise performance, and increase VEGF serum protein levels. Administration of the NAD+ precursor, NMN, to 18-month-old mice restored the number of capillaries and capillary density of the old mice to the level in young mice. Moreover, they demonstrated that H₂S similar to NMN increases the level of SIRT1 and the combination of NAD precursor, NMN, and H₂S donor, NaHS, more potently increased capillary density and reduced the number of apoptotic ECs in vivo and physical endurance of mice than either treatment alone (Das et al., 2018). Interestingly, Tyshkovskiy et al. (2019) found that cystathionine-γ-lyase was one of the most significant commonly upregulated genes among 15 known lifespan-extending interventions in mice.

### 5.4 Polyamine metabolism

Similar to yeast, worms, and flies, in C57BL/6J female mice, supplementation with spermidine and spermine (but not putrescine) significantly extends median lifespan. Spermidine also significantly extends lifespan when supplementation is started late in life (starting at 18-month-old mice). Spermidine supplementation has cardioprotective effects, resulting in reduced cardiac hypertrophy and preserved diastolic function in old mice. These organ-level findings are associated with enhanced cardiac cell autophagy, mitophagy, and mitochondrial respiration and spermidine fails to provide cardioprotection in mice that lack Atg5, which is essential for autophagy in cardiomyocytes (Eisenberg et al., 2016). It will be of interest to determine how MetR affects the levels of polyamines in different tissues and whether the mechanisms of lifespan extension by MetR and supplementation with spermidine and spermine overlap. MetR can be expected to decrease the levels of spermidine and spermine since it decreases the level of dcSAM. However, MetR extends lifespan and causes a 10-fold increase in the polyamine spermidine in livers of Lmna progeroid mice (Barcena et al., 2018).
Although the effect of MetR on lifespan extension was reported in 1993, the mechanisms at play are not completely understood. Our recent finding that long-lived flies display higher levels of methionine suggests that regulation of lifespan depends on flux in the methionine cycle and levels of SAH/SAM, rather than absolute methionine levels. Although it is clear that each of the three branches of methionine metabolism—the methionine cycle, the transsulfuration pathway, and polyamine biosynthesis—plays a significant role in lifespan extension, how these branches crosstalk during regulation of lifespan is unknown. Moreover, how activity in these different branches of methionine metabolism change with age in different tissues and organs remains to be elucidated. \(^{13}\)C-metabolic flux analysis (MFA) is a useful approach to determine cellular metabolic flux (Jin et al., 2004; Sauer, 2006). With this method, cells/organisms are fed isotope-labeled nutrients, the labeling patterns of intracellular metabolites are measured, and computational methods are used to estimate flux (Zamboni, 2011). \(^{13}\)C-metabolic flux methods to comprehensively quantify metabolic flux through the multiple methionine-related pathways have been developed for mammalian cells (Shlomi, Fan, Tang, Kruger, & Rabinowitz, 2014). To achieve the strongest lifespan extension via manipulations of methionine metabolism, it will be important to quantify how metabolic flux through the multiple methionine-related pathways changes with age in specific tissues and organs. Moreover, a full understanding of MetR is likely to require the identification of specific methyltransferases affected by changes in SAM and SAH. Recently, the set of CpG sites, the so-called “epigenetic clocks,” has been used to predict chronological and biological age (Horvath, 2013); it will be interesting to ask if a specific signature of CpG sites predicts the MetR response. Furthermore, it will be of interest to explore whether noninvasive methods similar to Met-PET can be used to evaluate the activity of methionine metabolism in humans and to predict the potential benefits of MetR for lifespan extension. Finally, given the high therapeutic potential, it will be interesting to search for drugs that can mimic the MetR response and extend lifespan via reprogramming of methionine metabolism.

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**CONFLICT OF INTEREST**

None declared.

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