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Stimulating S-adenosyl-L-methionine synthesis extends lifespan via activation of AMPK

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Dietary restriction (DR), such as calorie restriction (CR) or methionine (Met) restriction, extends the lifespan of diverse model organisms. Although studies have identified several metabolites that contribute to the beneficial effects of DR, the molecular mechanism underlying the key metabolites responsible for DR regimens is not fully understood. Here we show that stimulating *S*-adenosyl-L-methionine (AdoMet) synthesis extended the lifespan of the budding yeast *Saccharomyces cerevisiae*. The AdoMet synthesis-mediated beneficial metabolic effects, which resulted from consuming both Met and ATP, mimicked CR. Indeed, stimulating AdoMet synthesis activated the universal energy-sensing regulator Snf1, which is the *S. cerevisiae* ortholog of AMP-activated protein kinase (AMPK), resulting in lifespan extension. Furthermore, our findings revealed that *S*-adenosyl-L-homocysteine contributed to longevity with a higher accumulation of AdoMet only under the severe CR (0.05% glucose) conditions. Thus, our data uncovered molecular links between Met metabolites and lifespan, suggesting a unique function of AdoMet as a reservoir of Met and ATP for cell survival.

S-adenosyl-L-methionine | *S*-adenosyl-L-homocysteine | calorie restriction | AMP-activated protein kinase | yeast

The two metabolic intermediates, *S*-adenosyl-L-methionine (AdoMet) and *S*-adenosyl-L-homocysteine (AdoHcy), are key intermediates of methionine (Met) metabolism (1). AdoMet-dependent transmethylation is central to the regulation of numerous biological processes, including metabolism, signal transduction, and gene expression (1). Recent studies have documented the contribution of AdoMet-dependent transmethylation to the modulation of the lifespan in yeasts, worms, and flies (2, 3). In transmethylation reactions, AdoMet is converted to AdoHcy, an inhibitor of methyltransferases (1).

There has been considerable interest in the ability of dietary restriction (DR) to both improve health and increase longevity (4–6). DR, such as calorie restriction (CR) or Met restriction, extends the lifespan of a wide range of species (4–9). Although we have knowledge of several metabolites that contribute to the beneficial effects of DR (10–12), the molecular mechanism underlying the key metabolites responsible for DR regimens is far from complete.

In this study, we examined how Met metabolites influenced the lifespan of yeast. We found that stimulating AdoMet synthesis, which consumes both Met and ATP, resulted in extended lifespan and was epistatic to CR. Indeed, stimulating AdoMet synthesis led to AMP-activated protein kinase (AMPK) activation and increased lifespan. Unexpectedly, we discovered a unique effect of AdoHcy: that is, that stimulation of AdoMet synthesis resulted in lifespan extension and was epistatic to CR.

Results

Identification of *SSG1-1* Mutants That Extended Lifespan. AdoMet is a central coenzyme in the metabolism that occurs in the majority of biological methylation reactions (1). AdoHcy is a competitive inhibitor of the methylation reactions catalyzed by the AdoMet-dependent methyltransferases. Therefore, the *Saccharomyces cerevisiae SAH1*, encoding AdoHcy hydrolase and hydrolyzing AdoHcy to adenosine and homocysteine (Fig. 1*A*), is an essential gene for cell growth. Previously we found that mutation of *sah1-1* slowed growth at all temperatures examined (25~37 °C) and led to the accumulation of AdoHcy and AdoMet (13) (Fig. 1 *B* and *C*). Because the homeostasis of cellular metabolism is closely linked to lifespan (14), we measured the chronological lifespan (CLS) by monitoring the survival periods of nondividing yeast cells that had passed the postdiauxic phase (15). As shown in Fig. 1*D*, the *sah1-1* mutant had a shortened CLS.

To obtain a new regulator of lifespan, we screened for suppression of the *sah1-1* growth defect at elevated temperatures (36–37 °C). In this screening, 15 intragenic suppressors in the *sah1-1* gene were obtained. The remaining 101 suppressor mutants had dominant mutations that could be classified into one complementation group, designated *SSG1*, for the spontaneous suppression of growth-delay in *sah1-1* (Fig. S1*A*). One of these mutants, designated *SSG1-1*, was chosen for further study. Cloning and sequencing of the gene that suppressed the slow-growth of the *sah1-1* strain suggested that the *SSG1-1* mutation was an allele of the *YHR032W* (Fig. S1 *B* and *C*). *YHR032W* has high sequence similarity with the multidrug and toxin extrusion family of transporters, and overexpression of the *YHR032W* (designated as *ERC1*) in the *S. cerevisiae* DKD-5D-H strain background confers ethionine resistance and AdoMet accumulation. However, the

Significance

Calorie restriction (CR) or methionine (Met) restriction extends the lifespan of diverse model organisms. Here we carefully examined how Met metabolites influenced aging in yeast. We showed that stimulating *S*-adenosyl-L-methionine (AdoMet) synthesis, which consumes both ATP and Met, resulted in an extended lifespan and was epistatic to CR. Indeed, stimulating AdoMet synthesis led to AMP-activated protein kinase activation and increased lifespan. Furthermore, we revealed an effect of *S*-adenosyl-L-homocysteine that contributed to longevity with a higher accumulation of AdoMet. The most common CR regimen involves reducing caloric intake, an unpopular trade-off. We have shown that stimulating AdoMet synthesis per se in yeast could produce physiological conditions that mimicked CR.

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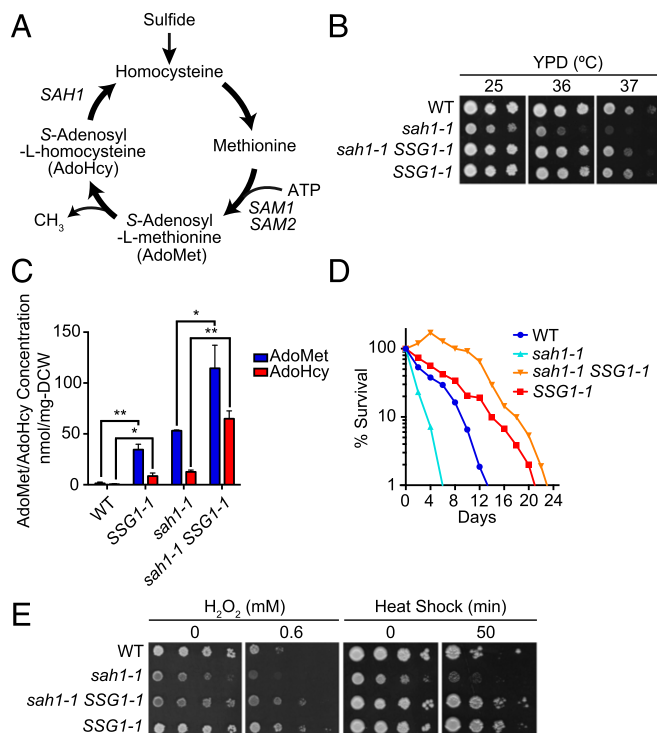

Fig. 1. *SSG1-1*, a dominant mutation, suppressed slow growth of the *sah1-1* strain and extended CLS. (*A*) Schematic diagram of methionine metabolism. (*B*) Growth of various strains on solid YPD medium. Serial dilutions of cells were spotted onto the plates, which were then incubated for 2~3 d at 25 °C, 36 °C, or 37 °C. (*C*) Intracellular AdoMet and AdoHcy levels. Mean ± SD (n = 3); $^{*}P < 0.05$; $^{**}P < 0.01$ (t test, two-tailed, parametric unequal variance). (*D*) CLS curve. (*E*) Hydrogen peroxide stress and heat-shock stress tests. Tenfold serially diluted cells were spotted onto solid medium containing 0.6 mM hydrogen peroxide (SDC plates at 25 °C) or were subjected to heat-shock treatment at 55 °C in YPD plates, which were then transferred to 25 °C and incubated for 3 d.

details of its function are unknown (16, 17). Although the *SSG1-1* mutants showed resistance to ethionine, overexpression of the *YHR032W* (*W303YHR032W*) in our strain background (W303-1A) failed to increase ethionine resistance (Fig. S1*D*). Moreover, neither deletion nor overexpression of *W303YHR032W* could suppress the slow growth of the *sah1-1* mutant (Fig. S1*E*). A comparison of the *SSG1-1* and *W303YHR032W* sequences revealed the presence of a single-base deletion at any one of the seven adenine nucleotide (A) repeats at a position between +1628 and +1634 of the coding region (Fig. S1*F*). This frameshift mutation was predicted to yield a protein with its C-terminal sequences altered from position 545, resulting in a protein that was 36 amino acids longer relative to Yhr032w. Because the insertion of an A at the same position failed to suppress the slow growth of the *sah1-1* strain (Fig. S1*G*), the A-deletion mutation was suggested to be responsible for the observed phenotypes. Interestingly, we found that the *SSG1-1* had the identical sequences with the *ERC1* gene (Fig. S1*F*). The nature of the *SSG1-1* may explain the gain-of-function in these phenotypes over the *W303YHR032W* allele. For this reason, we used the *SSG1-1* mutant allele in most of the subsequent experiments.

The growth defect of *sah1-1* can be suppressed by exogenous AdoMet but not AdoHcy (13), and overexpression of the *ERC1* results in the accumulation of AdoMet (16, 17). Therefore, the ability of the *SSG1-1* mutation to suppress the slow growth of the *sah1-1* strain led us to hypothesize that AdoMet levels might be increased in the *sah1-1 SSG1-1* cells. To test this possibility, we measured AdoMet levels in cell extracts by performing capillary liquid electrophoresis analysis. As anticipated, the *sah1-1 SSG1-1* and *SSG1-1* mutants contained higher levels of AdoMet than *sah1-1* and WT, respectively (Fig. 1*C*). We found that the AdoHcy levels were also increased in the presence of an *SSG1-1* mutation (Fig. 1*C*). To investigate whether the *SSG1-1* mutation could suppress the short-lived characteristic of *sah1-1* cells, we measured the CLS (Fig. 1*D*). Indeed, not only *sah1-1 SSG1-1* cells but also *SSG1-1* single mutants were shown to have a longer CLS, suggesting that the Ssg1-1 protein might play a role in longevity. Because long-lived mutants are occasionally more resistant to oxidative and thermal stress (18), we examined the stress resistance of the mutant strains and found that *SSG1-1* cells showed increased stress resistance (Fig. 1*E*). Therefore, we speculated that *SSG1-1* and AdoMet might be linked to longevity.

Stimulating AdoMet Synthesis Resulted in Extended Lifespan. The AdoMet synthetase genes (*SAM1* and *SAM2*) of *S. cerevisiae* catalyze the biosynthesis of AdoMet (1) (Fig. 1*A*). Because AdoMet levels were elevated in *SSG1-1* cells, we determined if CLS extension by *SSG1-1* was mediated by AdoMet synthesis in the strains deleted for the *SAM1* and *SAM2* encoding AdoMet synthetase. As the growth of *sam1*Δ *sam2*Δ cells is synthetic lethal (1), we constructed *SSG1-1 sam1*Δ and *SSG1-1 sam2*Δ double-mutant strains. The AdoMet levels were lower in both the *SSG1-1 sam1*Δ and *SSG1-1 sam2*Δ compared with the level in the *SSG1-1* cells (Fig. 2*A*). Indeed, the extended CLS of *SSG1-1* was eliminated by the deletion of either *SAM1* or *SAM2* (Fig. 2*B*). We next tested whether indeed AdoMet synthesis extended yeast CLS. We performed aging experiments using WT cells carrying an extra copy of the *SAM1* and *SAM2* genes from the *GAL1* promoter integrated into its genome. Strikingly, overexpressing *SAM1* and *SAM2* in WT cells significantly extended their CLS (Fig. 2*C*), concomitant with a high accumulation of AdoMet (Fig. 2*D*).

On the other hand, supplementing the medium with AdoMet was unable to extend the CLS of the WT cells (Fig. 2*E*). In contrast, extracellular AdoHcy contributed to an extended CLS with a higher accumulation of AdoMet than of AdoHcy (Fig. 2 *E* and *F*). It should be noted that we could not detect the AdoMet or AdoHcy levels in the culture medium in this experiment. In contrast, externally added AdoHcy had no effect on the CLS of *sam1*Δ (Fig. 2*G*), in which the increase in their AdoMet level was not more than that of AdoHcy (Fig. 2*H*). Taken together, these data showed that CLS extension by *SSG1-1* was dependent on AdoMet synthesis and suggested that stimulating AdoMet synthesis per se could promote longevity.

Stimulating AdoMet Synthesis-Mediated Longevity Is Epistatic to CR. AdoMet synthesis requires both Met and ATP (Fig. 1*A*). In *SSG1-1* cells, which can consume Met and ATP during chronological aging, higher amounts of AdoMet accumulated (Fig. 1*C*). All organisms produce ATP by glycolysis through the degradation of glucose. Met or glucose restriction was reported to cause lifespan extension in many eukaryotes (4–9). Thus, we assumed that consumption of Met and glucose for AdoMet synthesis was required for extended CLS in *SSG1-1* and that the CLS of *SSG1-1* would be diminished by limiting either the Met or glucose concentration in the medium. Indeed, we found that with Met-depletion or when the glucose concentration was limited to 0.05%, the CLS of *SSG1-1* could not be extended (Fig. 2 *I* and *J*). On the other hand, we found that treatment with 0.05% glucose slightly but reproducibly increased the maximum CLS of the WT (Fig. 2*J*). These results support the idea that consumption of Met and ATP in the *SSG1-1* cells contributed to the extended CLS. It should be noted that whereas *SSG1-1* is a methionine-prototrophic strain, the CLS of *SSG1-1* was not extended when Met was depleted from the medium, suggesting that the intake of

Fig. 2. Increased AdoMet production by AdoMet synthetase extended CLS. (*A*) The AdoMet and AdoHcy contents were measured. Mean ± SD ($n = 3$); ns, not significant; *$P < 0.05$; **$P < 0.01$ (*t* test, two-tailed, parametric unequal variance). (*B*) CLS curve. (*C* and *D*) Effect of overexpression of *SAM1* and *SAM2* in WT on CLS (*C*) and on AdoMet and AdoHcy levels (*D*). Cells in SGC medium (+ 2% galactose medium, *GAL1* promoter turned on) were measured. ns, not significant; ***$P < 0.001$ (*t* test, two-tailed, parametric unequal variance). (*E–H*) Effect of AdoMet or AdoHcy administration on CLS (*E* and *G*) and on AdoMet and AdoHcy levels (*F and H*) of WT or *sam1Δ* cells. Intracellular AdoMet and AdoHcy levels were quantified. Mean ± SD ($n = 3$); ns, not significant; **$P < 0.01$; ***$P < 0.001$ (two-way ANOVA with Tukey's multiple comparisons test, *F* and *H*). (*I–K*) *SSG1-1* cells required Met or glucose for longevity. CLS curves are shown. Met (*L*) and ATP levels (*M*) of cells are shown. ns, not significant; **$P < 0.01$ (*t* test, two-tailed, parametric unequal variance). (*N*) The AdoMet and AdoHcy contents were measured. Mean ± SD ($n = 3$); **$P < 0.01$; ***$P < 0.001$ (*t* test, two-tailed, parametric unequal variance).

extracellular Met was required for lifespan extension. We asked whether Met and ATP levels were decreased in the *SSG1-1*. Indeed, Met levels in the *SSG1-1* cells were lower than those in the WT cells (Fig. 2*L*). Unexpectedly, however, *SSG1-1* cells showed increased ATP levels (Fig. 2*M*), suggesting that *SSG1-1* might act to stimulate AdoMet synthesis by amplifying ATP levels.

A 0.5% glucose concentration has been used for CR studies in yeast, resulting in an extended CLS (15). We noted that the CLS of the *SSG1-1* strain, when grown on 0.5% glucose, was equivalent to that of the WT strain (Fig. 2*K*), suggesting that a common pathway may underlie the regulatory mechanism for longevity between CR and the *SSG1-1*–mediated mechanism.

To investigate the basis for stimulating AdoMet synthesis-mediated longevity, we conducted DNA microarray analysis using *SSG1-1* and WT cells (Table S1). We identified genes whose expression was increased more than twofold in the *SSG1-1* cells. In total, 21 genes were induced more than twofold, which included those involved in Met biosynthesis and inorganic phosphate metabolism, consistent with the high production of AdoMet (19) in the *SSG1-1* cells (Table S1). In addition, we found that

high-affinity glucose-transporter *HXT* genes and glucose-metabolism genes, which are associated with CR (20) (0.5% glucose), were significantly up-regulated (Table S1). Thus, these data provide further evidence that *SSG1-1* and CR shared a common pathway to confer longevity.

Stimulating AdoMet Synthesis Led to AMPK Activation. Snf1 is the central component of the glucose-repression pathway and is orthologous to the mammalian AMPK (21). Thus, we anticipated that Snf1 would be activated in the *SSG1-1* cells. Snf1 activity can be monitored by the phosphorylation of residue Thr210 of Snf1 (22). Compared with that in WT cells, phosphorylation of Thr210 was increased in *SSG1-1* cells (Fig. 3*A*), supporting the hypothesis that Snf1 was indeed activated. We confirmed that stimulating AdoMet synthesis modulated the activity of Snf1. We found that overexpression of *SAM1* and *SAM2* did cause an increase in Snf1 phosphorylation (Fig. 3*B*).

Snf1 activity correlates with a high ADP:ATP ratio (23); however, Snf1 was activated in the *SSG1-1* cells even though the ATP level was increased (Fig. 2*M*). These seemingly paradoxical results led us to examine the effect of *SNF1* deletion on ATP levels in the *SSG1-1* cells. We found that ATP levels were decreased in the *SSG1-1 snf1*Δ mutants (Fig. 2*M*), suggesting that Snf1 enhanced processes that generate ATP or inhibit others that consume ATP when cells are stimulate to have higher AdoMet levels, as in the *SSG1-1* cells. Interestingly, we found that AdoMet levels were reduced in the *SSG1-1 snf1*Δ mutants (Fig. 2*N*). Together, these data suggest that Snf1 was activated by stimulating AdoMet synthesis, which contributed to sustaining AdoMet levels by amplifying ATP (Fig. 3*D*). This mechanism of action is the subject of future investigations.

To test the role of *SNF1* in CLS, we generated *SSG1-1 snf1*Δ mutants. The CLS of the double mutant was decreased compared with that of the *SSG1-1* (Fig. 3*C*), suggesting that Snf1 might have been required for extending the CLS of *SSG1-1*. However, because the *SNF1* single-deletion mutants showed a shortened CLS (Fig. 3*C*), it is difficult to conclude that Snf1 mediated the CLS extension in *SSG1-1*. These results suggest that the beneficial effect of stimulating AdoMet synthesis was exerted, at least in part, through the activation of Snf1 (Fig. 3*D*).

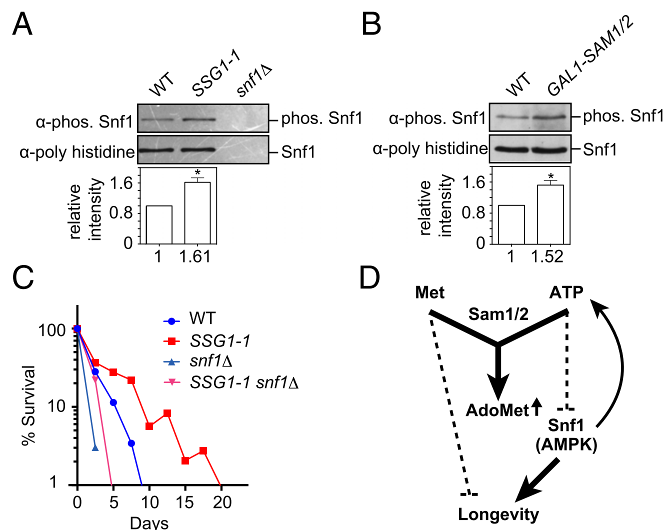

Fig. 3. *SSG1-1*- or stimulated AdoMet synthesis-mediated longevity associated with activation of AMPK. (*A*) Phosphorylation levels of Snf1 residue Thr210. Phosphorylation levels were quantified by ImageJ, and relative intensity normalized to total Snf1 is indicated below each band. Mean ± SD ($n = 3$); *$P < 0.05$ (*t* test, two-tailed, parametric unequal variance). (*B*) Effect of overexpression of *SAM1* and *SAM2* on Snf1 phosphorylation levels. Phosphorylation levels were quantified by ImageJ, and relative values normalized to total Snf1 are indicated below each band. Mean ± SD ($n = 3$); *$P < 0.05$ (*t* test, two-tailed, parametric unequal variance). (*C*) CLS curve is shown. (*D*) Model for stimulated AdoMet synthesis-mediated longevity in yeast.

Physiological Roles of AdoMet and AdoHcy in Lifespan. What is a physiologically relevant stimulus for AdoMet synthesis? *Schizosaccharomyces pombe* cells growing in medium containing a low glucose concentration of around 2.2 mM (about 0.04%) accumulate AdoMet (24); and Snf1 activity positively correlates with severe CR (0.05% glucose) (22). Thus, we hypothesized that cells faced with severe CR are channeled into AdoMet synthesis, which would be beneficial for longevity. We therefore checked whether the amount of AdoMet would be increased in the case of severe CR. Consistent with the previous report on *S. pombe*, *S. cerevisiae* WT cells also showed a significantly increased AdoMet level upon severe CR (0.05% glucose) (Fig. 4*A*). This severe CR increased the maximum CLS of WT cells compared with that for the control cells (2% glucose) (Fig. 4*C*). We further found that the AdoHcy level was also increased upon severe CR (Fig. 4*B*), which contributed to the accumulation of AdoMet as observed in Fig. 2*E*. In contrast, the amount of AdoMet and AdoHcy in *sam1*Δ cells upon severe CR remained unchanged (Fig. 4 *D* and *E*), and these cells showed a CLS similar to that of the controls (2% glucose) (Fig. 4*F*). Taken together, these data indicate that stimulation of AdoMet synthesis in cells under severe CR contributed to the maintenance of cell viability. Because *SSG1-1* mutants and severe CR-treated cells contained AdoHcy, these results further suggested that AdoHcy triggered the stimulation of AdoMet synthesis, leading to an extended CLS under these physiological conditions.

Although, glucose starvation (0% glucose) caused a significantly increased AdoMet level in WT cells (Fig. 4*A*), no beneficial effect of this increase on CLS was observed (Fig. 4*C*). Furthermore, CR (0.5% glucose) caused the most beneficial effect on longevity in WT and *sam1*Δ cells (Fig. 4 *C* and *F*), although AdoMet and AdoHcy synthesis was not stimulated (Fig. 4 *A*, *B*, *D*, and *E*). Thus, the contribution of stimulation of AdoMet synthesis to the longevity appears to be specific to the severe CR condition.

Discussion

Recent studies have documented that AdoMet-dependent transmethylation modulates the lifespan in yeasts, worms, and flies (2, 3). Here, we presented evidence that stimulating AdoMet synthesis turned on a metabolic switch that led to enhanced stress resistance and longevity. We identified a dominant mutation, *SSG1-1*, in the *YHR032W* as an allele for longevity. Interestingly, as far as we have examined, we found that every industrial yeast (e.g., *sakeYHR032W* in the *sake* yeast Kyokai no. 7 strain and *ERC1* in the DKD-5D-H, which is a hybrid strain between *sake* and laboratory yeast) has the same sequence of *SSG1-1* (16, 25), whereas laboratory yeasts (e.g., W303, S288C, and FL100, except for Σ1278b) do not have an adenine nucleotide deletion (Fig. S1*F*). *Sake* yeasts accumulate AdoMet at higher levels than laboratory yeasts (26), suggesting that it might be advantageous to reserve Met and ATP in the form of AdoMet to confer stress resistance and longevity to overcome periods of excess glucose/ATP that occur during fermentation. We speculate that yeasts in their natural environment are faced with variable carbon supplies and, therefore, accumulate AdoMet as a stable energy and nutrient source. In contrast, we propose that the laboratory yeast *YHR032W* locus lost its function during evolution because of the proper/controlled amount of nutrients that promoted cell growth. In this regard, it would be worth noting that the *SSG1-1* mutation could be readily generated by a single-base deletion at any one of the seven repeats of the adenine nucleotide and, conversely, that the reverse mutations would be facilitated by the insertion of a

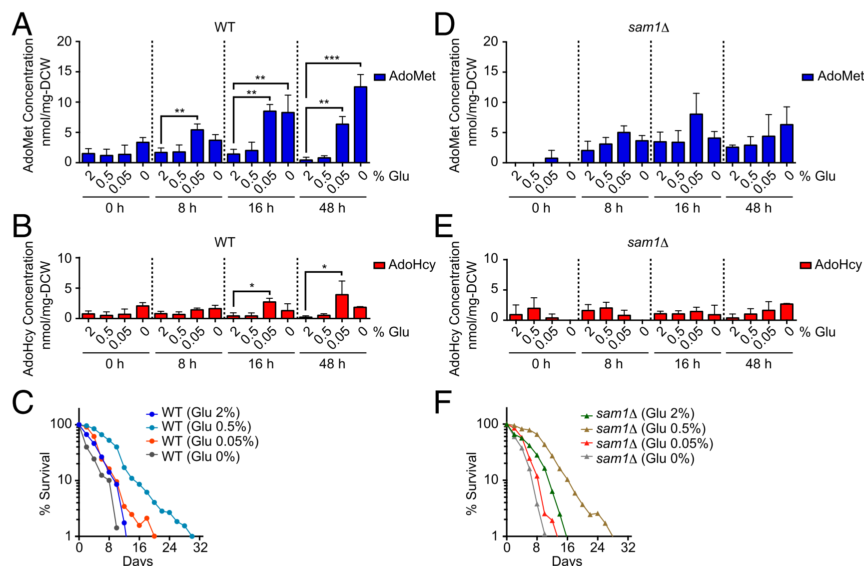

Fig. 4. Severe CR (0.05% glucose) promoted AdoMet production dependent on AdoMet synthetase, leading to extended maximum CLS. Intracellular AdoMet (*A* and *D*) and AdoHcy levels (*B* and *E*) or CLS (*C* and *F*) under several glucose-limited conditions are shown. Intracellular AdoMet and AdoHcy levels were quantified. Mean ± SD ($n = 3$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (one-way ANOVA with Tukey's multiple comparisons test; *A*, *B*, *D*, and *E*).

single adenine nucleotide to the repeated sequences in response to environment changes.

Christopher et al. proposed that the AdoMet:AdoHcy ratio is important, and if the ratio exceeded a threshold value, there was no growth inhibitory effect (27). Consistent with their observations, we found that the inhibitory effect of AdoHcy was abrogated in the simultaneous presence of AdoMet (13). However, it seems difficult to explain the negative or positive physiological effects because of cellular accumulation of AdoMet and AdoHcy simply in terms of the AdoMet:AdoHcy ratio. The accumulation of AdoHcy per se appears to inhibit cell growth and increases stress sensitivity in the *sah1-1* strain (13). In contrast, although the AdoMet:AdoHcy ratio in the *SSG1-1* strain is apparently similar to that of the *sah1-1* strain, the cell growth was WT level (stress resistance was better than WT).

Why does *SSG1-1* but not the *sah1-1* mutant extended CLS, despite the accumulation of AdoMet and AdoHcy in both strains (Fig. 1 *C* and *D*)? We demonstrated that deletion of *SAM1* or *SAM2* in *SSG1-1* reduced the AdoMet levels, leading to a loss of the beneficial effect on CLS (Fig. 2 *A* and *B*). The *sah1-1* strain also showed a reduced level of AdoMet when the *SAM1* or *SAM2* was deleted (Fig. S2*A*), suggesting that *sah1-1* and *SSG1-1* accumulated AdoMet by the same mechanism. However, the CLS of *sah1-1* cells was unaffected by the deletion of *SAM1* or *SAM2* (Fig. S2*B*). Therefore, some additional mechanisms appeared to exist on the effects of AdoMet/AdoHcy on lifespan extension. It was reported that elevated levels of AdoMet are detrimental to yeast if the excess AdoMet cannot be sequestered in their vacuole (28). Thus, the benefical effect of *SSG1-1* on lifespan may be explained by the accumulation of AdoMet and AdoHcy in the yeast vacuole. This possibility warrants further study.

We noted that deleting *SAM1* or *SAM2* slightly extended the maximum CLS compared with that of the WT (Fig. 2*B*), consistent with the observations that knocking down *sams-1* (AdoMet synthetase of *Caenorhabditis elegans*) by RNA interference in adults increases the lifespan of *C. elegans* and *sams-1* loss-of-function mutants have an increased lifespan (29, 30). Because Sam1/2 is required for Met and ATP to synthesize AdoMet and *sam1*Δ *sam2*Δ double-mutant cells show AdoMet auxotrophy (1), decreased methylation capacity (*sam1*Δ or *sam2*Δ) may allow a cell to sense a Met or ATP restriction. Indeed, we found that Snf1 activity was elevated in the *sam1*Δ or *sam2*Δ cells (Fig. S3).

At this point, it is unknown why CR (0.5% glucose) caused the most beneficial effect on longevity, although glucose starvation (0% glucose) had the detrimental effect on CLS (Fig. 4*C*). Previous study showed that CR maintains consistent ATP levels by enhanced mitochondrial metabolism during the chronological aging (31). Consistent with this finding, we found that *SSG1-1* cells showed increased ATP levels during the exponential growth phase (Fig. 2*M*). By contrast, acute glucose starvation may not be able to maintain ATP levels. It is possible that there may be a certain threshold of concentrations of glucose that maintained mitochondrial function contributing to extend CLS of yeast.

Our previous study proposed that the elevation of the cellular AdoMet level in *sah1-1* cells would seem to be the result of the activation of an unidentified compensatory mechanism to alleviate the inhibitory effect of AdoHcy on cell growth (13). In the work reported here, we shed additional light on the effect of AdoHcy in yeast. We noted that when *SAM1/2* was overexpressed or AdoMet was administered, the cells did not accumulate AdoHcy (Fig. 2*D* and Fig. S4). In contrast, administration of AdoHcy caused stimulation of AdoMet synthesis in WT (Fig. 2*F*), as in the *sah1-1* cells. Moreover, externally added AdoHcy, but not AdoMet, activated Snf1 (Fig. S5), resulting in lifespan extension (Fig. 2*E*). Thus, an unidentified compensatory mechanism—along with Snf1-activation—to alleviate the inhibitory effect of AdoHcy on cell growth will be important for this lifespan extension.

AdoHcy has received much attention as a risk factor for many diseases, including vascular and neurodegenerative diseases (32). Our present findings of this recently recognized effect of AdoHcy may provide new insights into the role played by AdoHcy in our health. Many of the effects of DR on longevity in model organisms have been linked to reduced protein and amino acid intake (4–6). We have shown here that stimulating AdoMet synthesis per se in yeast could produce physiological conditions that mimicked CR. In mammals, the CR benefits include reduced morbidity of a host of diseases, such as cancer and diabetes (5). Thus, developing new interventions that stimulate AdoMet synthesis could delay age-related disorders and may even have therapeutic potential in the treatment of Alzheimer's disease.

Materials and Methods

Details on materials and methods are in *SI Materials and Methods*. See Table S2 for *P* values for CLS analysis.

Yeast Strains and Media. The following yeast strains used in this study were all derivatives of W303: W303-1A (WT; *MAT***a** *trp1-1 leu2-3 ade2-1 ura3-1 his3-11 can1-100*), YMM222 (*MAT***a** *sah1-1*), YRT15 (*MAT***a** *SSG1-1*), YRT3 (*MAT***a** *sah1-1 SSG1-1*), YTO29 (*MAT***a** *sam1Δ::kanMX4*), YTO35 (*MAT***a** *sam2Δ::kanMX4*), YTO33 (*MAT***a** *SSG1-1 sam1Δ::kanMX4*), YTO39 (*MAT***a** *SSG1-1 sam2Δ::kanMX4*), YTO66 (*MAT***a** *sah1-1 sam1Δ::kanMX4*), YTO67 (*MAT***a** *sah1-1 sam2Δ::kanMX4*), YTO78 (*MAT***a** *GAL-SAM1 GAL-SAM2*), YTK22 (*MAT***a** *snf1Δ::kanMX6*), YTK23 (*MAT***a** *SSG1-1 snf1Δ::kanMX6*), YRT1 (*MAT***a**/α), YRT2 (*MAT***a**/α *sah1-1/sah1-1*), and YRT12 (*MAT***a**/α *sah1-1 SSG1-1/sah1-1*). Gene disruption was performed by a standard PCR-based method (33). Media used were as described previously (34).

CLS Assay. Yeast CLS analysis was performed in liquid SDC or SGC media (defined below), as previously described (35). Briefly, SDC or SGC cultures grown overnight were diluted (2×10^6 cells/mL) in fresh SDC or SGC media and incubated at 28 °C with shaking at 180 rpm. Viability was measured by plating aging cells onto YPD plates and monitoring CFUs starting from day 3, which was considered to be the initial survival (100%). All data were represented as the average of three independent experiments conducted at the same time. At least two sets of CLS experiments were performed with similar outcomes. CLS assays were performed with SDC medium [0.17% yeast nitrogen base without amino acids and ammonium sulfate (Difco); 0.5% (wt/vol) ammonium sulfate; 2% (wt/vol) glucose; amino acids to a final concentration of 20 mg/L (adenine, arginine, histidine, methionine, tryptophan, and uracil), 30 mg/L (isoleucine, leucine, lysine, and tyrosine), 60 mg/L (phenylalanine), and 150 mg/L (valine)]. SGC medium had the same composition as SDC, but contained 2% (wt/vol) galactose instead of glucose.

Microarray Analysis. RNA isolation and microarray profiling analyses were carried out as previously described (19, 36), using the Gene Chip Yeast Genome 2.0 Array (Affymetrix). Cells were grown to early log phase (2×10^6 cells/mL) in liquid SDC medium at 28 °C. A given gene was considered induced when its expression ratio was higher than 2.0. Microarray data have been deposited at the public repository Gene Expression Omnibus (GEO) under accession number GSE76206.

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