S-Adenosyl-L-homocysteine hydrolase in yeast: key enzyme of methylation metabolism and coordinated regulation with phospholipid synthesis

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Abstract ***S*-Adenosyl-L-homocysteine hydrolase (Sah1p, EC 3.3.1.1.) is a key enzyme of methylation metabolism. It catabolizes *S*-adenosyl-L-homocysteine, which is formed after donation of the activated methyl group of *S*-adenosyl-L-methionine (AdoMet) to an acceptor, and which acts as strong competitive inhibitor of all AdoMet-dependent methyltransferases. Sah1p is an essential enzyme in yeast and one of the most highly conserved proteins with up to 80% sequence homology throughout all kingdoms of life. *SAH1* expression in yeast is subject to the general transcriptional control of phospholipid synthesis. Profound changes in cellular lipid composition upon depletion of Sah1p support the notion of a tight interaction between lipid metabolism and Sah1p function.**

Keywords: *SAH1*; Phospholipid; Lipid; Methylation; Yeast

1. Introduction

S-Adenosyl-L-methionine (AdoMet) serves as the major methyl group donor for numerous highly specific methyltransferase reactions that involve a large variety of acceptor molecules, ranging from DNA, RNA and proteins to phospholipids and sterols [1–7]. In yeast and in higher eukaryotes, the major phospholipid, phosphatidylcholine (PtdCho), is synthesized de novo by three-step AdoMet-dependent methylation of phosphatidylethanolamine (PtdEtn). Ergosterol, the major yeast sterol, differs mainly in its side-chain from cholesterol present in mammals by an additional methyl group that is introduced by the AdoMet-dependent methyltransferase, Erg6p.

S-Adenosyl-L-homocysteine (AdoHcy), that is formed after donation of the activated methyl group of AdoMet to a methyl acceptor, is a strong competitive inhibitor of all AdoMet-dependent methyltransferases [1] (Fig. 1). In most species, *S*-adenosyl-L-homocysteine hydrolase (Sah1p) offers a single way for AdoHcy removal by hydrolysis to adenosine and homocysteine. Since this reaction is reversible, an increase in homocysteine levels may drive the formation of AdoHcy. According to its central role in controlling the methylation potential of the cell, Sah1p is a potential target for drugs of pharmacological interest, in particular in the context of methylation dependent viral replication. Clinical application, however, is limited due to the high cytotoxicity of Sah1p inhibitors.

In the yeast *Saccharomyces cerevisiae*, genes involved in lipid biosynthesis are subject to coordinated transcriptional regulation in response to the availability of lipid precursors, inositol and choline. These coregulated structural genes contain one or more copies of an inositol/choline response element, ICRE (UAS_{INO}), in their promoter regions [8], which mediate repression/de-repression in the presence or absence of lipid precursors, respectively. In a screening for genes which are regulated at the transcriptional level by inositol and choline [9], we have identified the yeast *SAH1* gene, encoding Sah1p. *SAH1* is an essential gene in yeast. Our data suggest that *SAH1* expression is coordinately regulated with genes involved in phospholipid biosynthesis. Sah1p depletion led to significant changes in lipid metabolism, resulting in accumulation of triglycerides.

2. Materials and methods

2.1. Strains and media

S. cerevisiae wild-type strain *W303D* (R.Rothstein) was used throughout this study. Strains MSH-100 (*W303D SAH1/sah1::URA3*) and YSH-100 (*W303D SAH1/sah1::LEU2*) heterozygous for the *sah1* null mutation were constructed as described below. Defined yeast media lacking or containing inositol (80 μM) and choline (1 mM), respectively, were prepared as described previously [10]. Methionine was supplemented at concentrations of 0.1 and 1 mM. *Escherichia coli* and yeast cells were grown under standard conditions [11].

2.2. Plasmid constructions and gene disruption

Plasmid pBlueScript SK$^-$ (Stratagene) was used for standard DNA manipulations. Plasmid p18.2 harboring a 1658 bp *Eco*RI–*Xho*I cDNA insert was isolated in a differential screening for yeast genes that are regulated at the transcriptional level by lipid precursors, inositol and choline [9]. Plasmids p18.2ΔURA3 and p18.2ΔLEU2 were constructed by replacing the *Hin*cII–*Hin*cII fragment of plasmid p18.2 by *URA3* or *LEU2* selectable markers that were isolated from plasmids pJH-U1 and pJH-L3 (provided by J. Hill), cut with *Hin*dIII and *Hin*dIII/*Sac*I, respectively, and blunt ended. Disruption of the *SAH1* chromosomal

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Abbreviations: AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine; Sah1p, *S*-adenosyl-L-homocysteine hydrolase; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine

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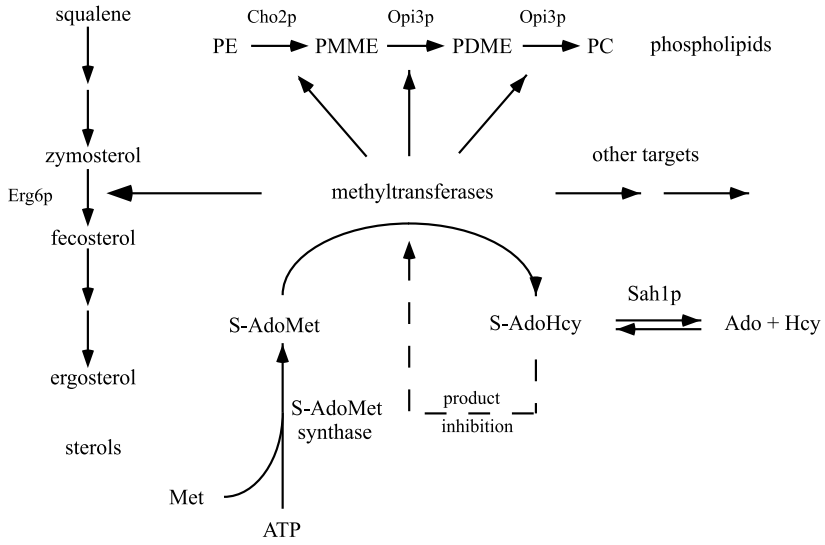


Fig. 1. Scheme of methylation metabolism and the role of Sah1p in lipid biosynthesis.

locus was carried out by transformation of the diploid wild-type strain *W303D* with *Aat*II–*Bgl*II fragments of plasmids p18.2ΔURA3 and p18.2ΔLEU2, followed by selection of transformants for *Ura*$^+$ or *Leu*$^+$ prototrophy [12]. Deletion of nucleotides 547 through 1042 of the *SAH1* reading frame was confirmed by PCR [11] of whole yeast cell extracts [13] using *SAH1* and marker gene specific primers. In both cases, the observed fragment length of the PCR products matched the predicted sizes for *URA3* and *LEU2* disruption, in addition to the fragment of the wild-type allele. The *SAH1* reading frame was put under control of the regulatable *GAL1* promoter by inserting the *Bam*HI–*Bcl*I fragment of plasmid *p18.2* into the *Bam*HI site of plasmid pYES2 (Invitrogen). The resulting pYES2-SAH1 vector was transformed into yeast strain YSH-100, yielding strain YSH-120. For constitutive expression, the *SAH1* reading frame was put under control of the *PGK* promoter by inserting the *Bam*HI–*Bcl*I fragment of plasmid p18.2 into the *Bgl*II site of vector pMA91 [14]. The resulting pMA91-SAH1 vector was transformed into strain MSH-100, yielding strain MSH-120.

2.3. RNA isolation and hybridization

Yeast total RNA was prepared by the hot phenol method [11] from wild type strain *W303D* grown to OD_{600} 1 in defined medium with differential supplementation of lipid precursors, inositol and choline, as indicated. Each 7 μg of total RNA was separated on a 1.2% agarose gel under denaturing conditions and transferred to uncharged nylon membranes (Qiagen) using a Posiblot^{TM} Pressure Blotter (Stratagene) in 20xSSC transfer buffer (3 M NaCl and 0.3 M Na-citrate, pH 7.0). After UV-crosslinking (Stratalinker^{R}1800, Stratagene), the membrane was probed with DIG-labeled *SAH1*-specific probe [11]. DIG-labeled *PMA1*-specific probe was used as the internal standard. Signals were detected using chemiluminiscent detection with CDP-Star^R (Roche) and quantified by densitometric scanning.

2.4. Preparation of cytosolic extracts

Yeast cells grown to mid-logarithmic phase (OD_{600} 1–2) in defined medium were harvested by centrifugation and resuspended in breaking buffer (10 mM Tris–HCl and 1 mM PMSF, pH 7.4) [11]. Cells were disrupted with glass beads in a Braun-Melsungen homogeniser under CO_2-cooling. The homogenate was centrifuged at $5000 \times g$ for 10 min and at $40\,000 \times g$ for 1 h at 4 °C. Protein concentrations were determined according to Lowry [15].

2.5. Enzyme assay

Sah1p activity was determined in the direction of AdoHcy synthesis [16]. The standard reaction mixture of 90 μl contained 50 mM Tris–HCl, pH 8.5, 0.5 mM adenosine, 0.1 μCi of [8-^{14}C]adenosine (59.8 mCi/mmol, Du Pont), 10 mM DL-homocysteine, 33 μM β-mercaptoethanol and 50–100 μg of enzyme preparation. Incubations were carried out at 30 °C. Aliquots were taken during 30 min of incubation, the reaction was stopped by addition of 10 μl of a 50 % (w/v) solution of trichloroacetic acid and the samples were kept on ice for 10 min. The protein precipitate was removed by centrifugation and the supernatant was stored at −20 °C until further use. For product identification, 10 μl reaction aliquots were separated on silica gel TLC plates (silica gel 60 F254, Merck) using as a solvent *n*-butanol/acetic acid/water, 12:3:5 (v/v/v). Zones containing AdoHcy were identified by scanning with a TLC-linear analyser Tracemaster 20 (Berthold Analytic Instruments), cut out and radioactivity determined by liquid scintillation counting.

2.6. Lipid analyses

Lipid extracts were prepared by the Folch method [17]. Phospholipids and neutral lipids were determined according to published procedures [18,19].

2.7. Miscellaneous procedures

DNA manipulation, plasmid transformation into *E. coli* and its retrieval from *E. coli* were performed following standard procedures [11]. Transformation of *S. cerevisiae* was carried out using the lithium acetate method [12]. DNA sequencing was performed using the dye-terminator method [20] on an ABI sequencer. Sequences were edited and analyzed using the GCG Wisconsin program package [21]. Comparison to nucleic acid sequence databases was performed using BLAST [22] and sequence alignments were computed using MACAW [23].

3. Results

3.1. Sequence analysis and evolutionary conservation of Sah1p

The *SAH1* structural gene was cloned in a screening designed to identify genes that are transcriptionally regulated by lipid precursors, inositol and choline [8,9]. Sequence analysis unveiled that the cloned cDNA fragment was identical to reading frame YER043c on chromosome V that was previously designated *SAH1*, based on its homology to *Leishmania donovani SAH* [24].

Multiple sequence alignments of the Sah1 proteins from different organisms including *Archaea* (*Sulfolobus solfataricus*), *Eubacteria* (*Rhodobacter capsulatus*), yeast (*S. cerevisiae*), slime mold (*Dictyostelium discoideum*) and higher eukaryotes

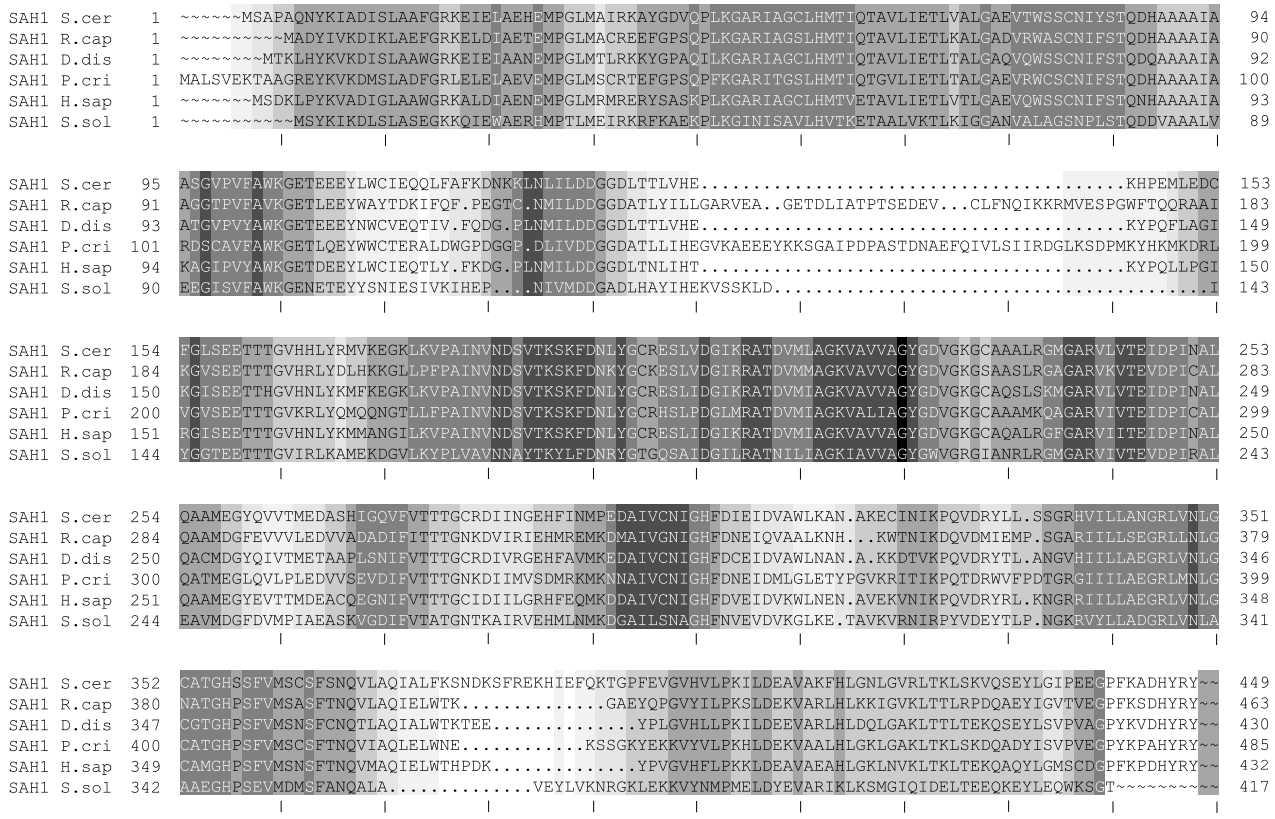


Fig. 2. Multiple sequence alignment of Sah1p from different organisms, including yeast *Saccharomyces cerevisiae – S .cer*; Eubacteria *Rhodobacter capsulatus – R. cap*; slime mold *Dictyostelium discoideum – D. dis*; plant *Petroselenium crispum – P. cri*; human *Homo sapiens – H. sap*; and Archaea *Sulfolobus solfataricus – S. sol* (10–15).

including plant (*Petroselenium crispum*), rat and human [25–30] showed overall sequence homology of about 80% (Fig. 2). Yeast and human SAH are about 70% identical; thus, Sah1p is among the 90 most highly conserved yeast proteins, including actin (90% identity), ubiquitin (90%), histones (70–90%), ribosomal (70%) and heat shock proteins (70%) [31].

3.2. Sah1p is an essential enzyme in yeast

To verify the identity of the cloned gene, gene disruptions by insertion of *URA3* or *LEU2* markers into the chromosomal *SAH1* locus were performed. The diploid wild-type strain *W303D* was transformed with linearized *SAH1*Δ*URA3* and *SAH1*Δ*LEU2* fragments of plasmids p18.2ΔURA3 and p18.2ΔLEU2, replacing the endogenous *SAH1* locus, and resulting in uracil and leucine prototrophy, respectively. Disruption was confirmed by PCR using *SAH1* and marker gene specific primers (data not shown).

Sah1p specific activity of the diploid disruptant strains heterozygous for the *SAH1* chromosomal locus was reduced by 40–50%, compared to the diploid wild-type control, reflecting the reduced gene copy number in this strain (data not shown). Upon dissection of diploid disruptant strain MSH-100 heterozygous for the *sah1* locus on YPD plates, only two spores which were uracil auxotrophs germinated and led to the formation of colonies (Fig. 3A). Spores containing the disrupted *sah1* locus either did not germinate or led to the formation of microcolonies of 10–100 cells, which ceased in their further growth. Neither supplementation with all amino acids (including 10 mM cysteine) nor homocysteine supplementation

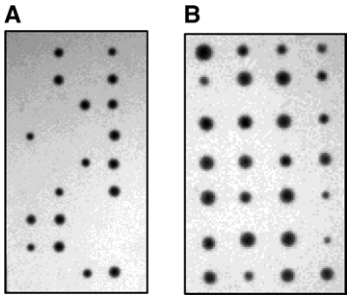


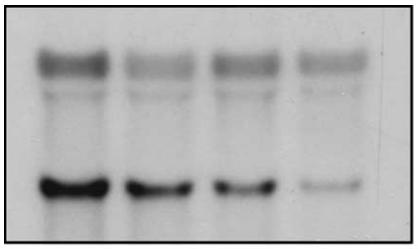
Fig. 3. Sah1p is an essential enzyme. Yeast tetrad analysis of (A) diploid disruptant strain MSH-100, heterozygous for the *sah1* chromosomal locus (*SAH1*/*sah1::URA3*), and (B) diploid disruptant strain MSH-120 (*SAH1*/*sah1::URA3*), harboring the cognate *SAH1* gene on plasmid pMA91.

restored growth of haploid *sah1::URA3* disruptant strains. In order to prove that the lethal phenotype resulted indeed from *SAH1* disruption, the cognate plasmid harboring the entire wild-type *SAH1* gene under control of the constitutive *PGK* promoter was transformed into the heterozygous diploid disruption strain MSH-100, prior to tetrad dissection. Appearance of four viable colonies on the dissection plate demonstrated that the cognate clone complemented the lethal *sah1* mutant phenotype (Fig. 3B). Continuous growth of these transformants under non-selective conditions (YPD medium) did not result in loss of the episomal plasmid, indicating that *SAH1* is not only essential for germination, but also for vegetative growth.

In order to investigate phenotypes associated with limited expression of *SAH1*, the gene was placed under the control of the regulatable *GAL1* promoter and transformed into strain YSH-100, heterozygous for the chromosomal *SAH1* locus (*SAH1/sah1::LEU2*). After sporulation, haploid strains with a deleted chromosomal allele of the *SAH1* gene and harboring the complementing plasmid were selected on galactose plates (strain YSH-121; *sah1::LEU2 pGAL1-SAH1*). After shift to glucose media, resulting in repression of *GAL1*-driven *SAH1* expression, strain YSH-121 grew very poorly and reached only 40% of the growth rate compared to cultivation in galactose-containing media. In the presence of 1 mM methionine in the growth medium, a large fraction of cells arrested in a peculiar terminal phenotype, characterized by altered morphology and multiple buds (Fig. 4). Staining with the DNA-specific fluorescence dye, DAPI (4′,6′ diamidino-2-phenylindole), indicated that nuclear division in these cells was completed, suggesting that *SAH1* depletion resulted in a block in a late stage of cytokinesis (data not shown).

3.3. *SAH1 is regulated at the transcriptional level in coordination with phospholipid biosynthetic genes*

Sequence analysis of the 5′ region of *SAH1* revealed that at position –242 a 5′-GCACGTGAT-3′ sequence matching the consensus UAS_{INO} sequence, 5′-GCATGTGAA-3′, is present. UAS_{INO} mediates regulation of phospholipid biosynthetic genes in response to lipid precursors, inositol and choline [8]. In order to analyse the potential coregulation with genes involved in phospholipid biosynthesis, we have measured *SAH1* steady state mRNA levels in wild-type cells grown in the presence or absence of inositol (80 μM) and/or choline (1 mM). As shown in Fig. 5, *SAH1* was repressed in the presence of inositol by 20%, and was further repressed upon addition of choline to inositol-containing medium by 86%, compared to unsupplemented conditions. Choline alone had an intermediate repressing effect on *SAH1* expression. The repression of *SAH1* in response to inositol and choline availability was in the range of other genes involved in phospholipid synthesis [32]. Thus, Sah1p is regulated at the transcriptional level in coordination with genes involved in phospholipid metabolism, most likely through the consensus UAS_{INO} sequence. This differential regulation by lipid precursor supplementation is also reflected at the level of enzyme activity (Fig. 5). Interestingly, *SAH1* was not picked up in a recent micro array study to identify genes transcriptionally controlled by inositol, and by Ino2/Ino4 and Opi1 regulatory factors [33]. The lack of detection of *SAH1* cDNA on gene arrays may be due to the low expression level and/or different cultivation conditions prior to RNA extraction.



-	+	-	+	Inositol, 80 μM
-	-	+	+	Choline, 1 mM
100	80	48	14	*SAH1* mRNA, %
100	60	75	50	Sah1p activity, %

Fig. 5. Regulation of Sah1p at the levels of gene expression and enzyme activity in wild-type strain *W303D* grown to $OD_{600} = 1$ in the absence or presence of lipid precursors, inositol and choline, as indicated. *PMA1* mRNA was used as an internal standard (3.5 kb, upper band). Enzyme activity was measured as described in Section 2. Assays were performed in duplicate.

The UAS_{INO} sequence of the *SAH1* gene (5′-GCACGTGA-3′) overlaps with the consensus UAS_{MET} sequence, 5′-TCACGTGA-3′ that mediates regulation of genes whose products are involved in sulfur assimilation in response to methionine/AdoMet availability [34]. Thus, we investigated whether methionine supplementation also affected *SAH1* transcription. Supplementation of the growth medium with 0.1 or 1 mM methionine in the absence of lipid precursors, inositol and choline, did not change *SAH1* steady state mRNA levels (data not shown). However, Sah1p activity levels were reduced by 25% or 60% upon growth in the presence of 0.1 or 1 mM methionine, respectively, compared to unsupplemented conditions. These data suggest that regulation of Sah1p by methionine occurs at the post-transcriptional level.

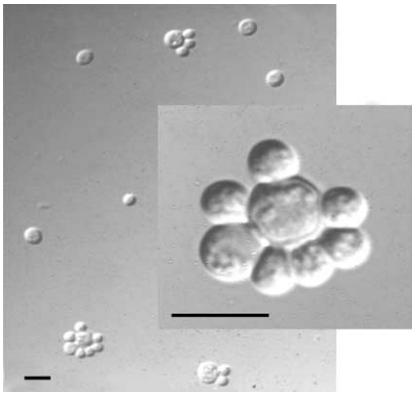


Fig. 4. Depletion of Sah1p in the *sah1* disruption strain YSH-120 (*SAH1/sah1::LEU2*), expressing *SAH1* under control of the *GAL1* promoter, results in a multiple budding phenotype in the presence of glucose and 1 mM methionine.

3.4. *Modulation of SAH1 expression affects lipid composition*

Upon conditions of *SAH1* repression in the absence of methionine, we observed that lipid droplets accumulated in strain YSH-121 already during logarithmic growth, a feature normally observed in later stages of growth in wild-type cells. Cell volume increased and frequent cell lysis occurred upon extended cultivation on glucose. This observation prompted us to analyze phospholipid, triacylglycerol, sterol and steryl ester content under conditions of Sah1p depletion in various stages of growth. Strain YSH-121 was cultivated with glucose as carbon source and collected at early-log, mid-log and late-log phases of growth. Under these conditions, biosynthesis of all lipid classes was significantly altered. Depletion of *SAH1* in the presence of glucose resulted in significantly reduced content of total phospholipids in an early phase of growth ($OD_{600} = 1$), whereas total sterols and triacylglycerol levels accumulated up to 2- and 5-fold, in comparison to wild type, during growth

Table 1
Deregulation of lipid synthesis in response to modulation of *SAH1* expression[a]

Strain/conditions	OD_{600}	Phospholipids, $\times 10^{-3}$ μg phosphorus/μg protein	Sterols, μg/μg phosphorus	Steryl esters, μg/μg phosphorus	Triacylglycerols, μg/μg phosphorus
Wild type W303, glucose	1	38.5 ± 0.3	3.1 ± 0.3	1.3 ± 0.7	1.7 ± 0.2
	5	46.3 ± 1.9	2.6 ± 0.5	1.3 ± 0.2	4.5 ± 0.2
	10	48.6 ± 2.6	2.7 ± 0.3	6.7 ± 0.3	9.7 ± 0.9
YSH-121, glucose	1	10.9 ± 1.2	3.1 ± 0.1	1.4 ± 0.6	7.9 ± 0.2
	5	45.5 ± 5.1	9.8 ± 0.9	3.9 ± 0.2	10.5 ± 2.3
	10	38.2 ± 0.2	4.9 ± 0.1	6.1 ± 0.4	18.6 ± 0.1

[a] The data represent two biological and two technical replicates shown as mean values ± S.E.

(Table 1). Increase in steryl ester and triacylglycerol levels upon depletion of Sah1p is in line with the observed accumulation of lipid droplets.

4. Discussion

AdoMet-dependent methyltransferases catalyze the bulk of methylation reactions in the cell. Regulation of the pool of AdoHcy, a strong product inhibitor of all AdoMet-dependent methyltransferases, is, therefore, of imminent importance [1,2]. In most organisms, AdoHcy is catabolized by Sah1p, which thus plays a pivotal role in regulating the methylation potential in the cell. Interference with Sah1p activity potentially affects methylation of different molecule classes, including phospholipids, sterols, DNA, RNA and proteins, and is associated with high cytotoxicity [1,2].

Sah1p has maintained its primary sequence – and presumably its function – throughout evolution. The bacterial *R. capsulatus* SAH (*ashY* gene product) shows one of the highest levels of conservation that has been observed between human and bacterial enzymes [26]. Yeast and human SAH are about 70% identical, and the yeast ortholog is thus among the 90 most highly conserved yeast proteins [31]. Consistent with its important metabolic function, we found that Sah1p is an essential enzyme in yeast, which is in line with previous findings resulting from a genome wide functional approach [35]. However, conflicting results exist on the viability of *sah1* disruptant strains derived from a study making use of Ty1 transposon insertions in genes of chromosome V of *S. cerevisiae* [36]. Also, in a recent study, *sah1* insertion mutants could be rescued by addition of cysteine or homocysteine to the growth media [37]: the *sah1* mutant was obtained by insertion of the *URA3* selection marker between nucleotides +1100 of the reading frame, and 197 base pairs downstream of the *SAH1* stop codon, which only eliminated the C-terminal 83 amino acids. Since the conserved regions of the enzyme are not included in this area, Sah1p function presumably was not completely eliminated in this mutant, resulting in a rather leaky phenotype that could be rescued by amino acid supplementation. However, use of this mutant in comparison to cells (conditionally) depleted of SAH may prove useful to dissect molecular mechanisms associated with cytotoxicity of SAH directed inhibitors, and metabolic consequences due to a lack of sulfur recycling. Consistent with an essential function of Sah1p in yeast, disruption of the *Ahcy* locus in the mouse results in early embryonic lethality [38]. Interestingly, disruption of the *ahcY* gene coding for *SAH* in the gram-negative bacterium *R. capsulatus* has no effect on viability [26] if methionine or homocysteine is provided, and numerous organisms lack SAH orthologs altogether. However, the existence in these cell types of an alternative system to degrade AdoHcy, i.e., by *S*-adenosylhomocysteine nucleosidase and *S*-ribosylhomocysteine hydrolase, underscores the physiological importance of AdoHcy removal from the cellular milieu.

Very recently, a temperature sensitive mutant allele of *SAH1*, *sah1-1*, was identified as a suppressor of the Ca^{2+} sensitive phenotype of *zds1* null mutants [39]: Zds1p is involved in chromatin silencing, aging and establishment of cell polarity, and the mutant is characterized by a growth defect, G_2 cell cycle delay and polarized bud growth. Accumulation of both AdoHcy and AdoMet in the *sah1-1* mutant was shown to result in repression of *SWE1* and *CLN2* gene expression, which are both involved in cell cycle control and upregulated in the *zds1* mutant. A similar, albeit less pronounced suppressing effect on the Ca^{2+}-sensitivity of *zds1* mutants was achieved by supplementation of cells with AdoMet or AdoHcy. Although the mechanisms by which these compounds downregulate *SWE1* and *CLN2* expression are not known, these data further underscore the importance of maintaining the AdoMet/AdoHcy ratio and, consequently, the cellular methylation potential, by Sah1p.

In yeast, most genes encoding enzymes of phospholipid biosynthesis are subject to regulation at the level of transcription in response to the availability of lipid precursors, inositol and choline. This transcriptional control is mediated through one or more copies of UAS_{INO} in the promoter regions of these genes, and is, in particular, dependent on the continuous synthesis of PtdCho [8]. The final three steps in PtdCho biosynthesis are catalyzed by AdoMet-dependent methyltransferases, Cho2p and Opi3p and may, thus, be sensitive to the AdoHcy/AdoMet ratio and Sah1p activity level in the cell. Identification of an inositol/choline response element (ICRE, UAS_{INO}) in the promoter region of the *SAH1* gene and its regulation in accordance with genes involved in phospholipid synthesis suggests a coordination of Sah1p activity level in the cell with ongoing phospholipid synthesis. Indeed, preliminary experiments suggest that overexpression of *SAH1* in partially methylation-deficient yeast strains (*cho2* or *opi3* mutants, defective in phosphatidylethanolamine *N*-methyltransferase, and phospholipid *N*-methyltransferase activities, respectively) stimulated residual methylation and suppressed to some extent phenotypes associated with these mutations (Tehlivets and Kohlwein, unpublished). Interestingly, in contrast to other genes involved in phospholipid synthesis, we found that *SAH1* expression was also significantly repressed

by choline. Incorporation of choline into PtdCho via the Kennedy-pathway provides an alternative route to the de novo pathway, which requires AdoMet-dependent methylation. The observed downregulation of *SAH1* expression by choline may reflect a reduced requirement for de novo PtdCho synthesis under conditions of choline supplementation.

The repression of Sah1p activity by methionine indicates additional coordinative regulation with enzymes involved in sulfur assimilation and AdoMet formation [34]. Thus, Sah1p potentially represents a convergence point of phospholipid and methionine specific regulation. The impact of Sah1p controlled methylation and phospholipid synthesis is also evident from data based on conditional *SAH1* expression. Not only PtdCho synthesis was found to be drastically affected upon Sah1p depletion. Both sterol and steryl esters accumulated significantly under these conditions, also reflecting interference of altered methylation potential with sterol formation, presumably at the level of Δ^{24}-sterol-C-methyltransferase (Erg6p). In fact, studies have demonstrated that structural analogs of AdoHcy inhibited Erg6p activity in vitro [40]. However, most significantly, depletion of *SAH1* led to an accumulation of triacylglycerols in the cell. These observations directly link activity of the enzyme to alterations in lipid homeostasis. Sah1p catalysis not only functions both in the direction of AdoHcy hydrolysis, but also in the reverse reaction, utilizing homocysteine and adenosine to form AdoHcy. It is tempting to speculate that one potential mechanism of homocysteine as a risk factor for inducing lipid alterations in humans is by altering the methylation potential of the cell through elevated Sah1p-dependent AdoHcy formation.

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