Involvement of S-adenosylmethionine in G1 cell-cycle regulation in Saccharomyces cerevisiae

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S-adenosyl-L-methionine (AdoMet) is a molecule central to general metabolism, serving as a principal methyl donor for methylation of various cellular constituents. The alteration in the availability of AdoMet has profound effect on cell growth. A mutant allele of Saccharomyces cerevisiae gene SAH1 encoding S-adenosyl-L-homocysteine (AdoHcy) hydrodase, was isolated as a mutation that suppressed the Ca2+-sensitive phenotypes of the zds1Δ strain, such as the Ca2+-induced, Swe1p- and Cln2p-mediated G1 cell-cycle arrest, and polarized bud growth. The mutation (sah1-1) led the cells to accumulate AdoMet besides AdoHcy, the substrate of Sah1p. The cells treated with exogenous AdoMet and AdoHcy had markedly decreased levels of SWE1 and CLN2 mRNA, providing the basis for the suppression of the Ca2+-sensitivity by the sah1-1 mutation. Exogenous AdoMet transiently led the cells to G1 cell-cycle delay whereas AdoHcy caused growth inhibition irrelevant to the cell cycle. The effect of AdoMet in inducing the cell-cycle delay was exerted in a manner independent of Met4p, an overall transcriptional activator for MET genes. Our observation provides an insight into the role played by AdoMet in cell cycle regulation.

Eukaryotic cells coordinate cell growth in response to a variety of external signals. In the budding yeast Saccharomyces cerevisiae, commitment to a new round of cell duplication occurs at a control point called Start. Execution of Start demands a sufficient level of G1 cyclin/Cdc28 protein kinase activity and is a requirement for DNA synthesis, bud formation, and replication of the spindle pole body (1).

Unver and Hertwell (2) found that sulfate starvation of a prototroph, methionine starvation of an auxotroph, or a shift of a conditional methionyl-tRNA synthetase mutant to a restrictive condition led to arrest in G1. They proposed that the signal for the nutrients was generated at the level of protein biosynthesis. It was recently shown that disruption of the gene for MET30, an E3 ubiquitin ligase, caused the arrest of G1 (3). MET30 is an essential gene that encodes a negative regulator of the sulfur amino acid biosynthesis pathway (4, 5). The uncontrolled accumulation of Met4p, a transcriptional activator for MET genes (6), in a met30Δ strain has been suggested to inhibit passage of Start by preventing the accumulation of G1 transcripts, such as CLN1 and CLN2 (3). It is therefore reasonable to expect some sort of connection between the cyclin–Cdc28p kinase complex and the metabolism of AdoMet in yeast.

Materials and Methods

Strains and Media. All yeast strains are derivatives of strain W303. The strains used were the following: DHT22-1b (MATa trpl leu2 ade2 ura3 his3 can1-100 W303-1A a gift from R. Rothstein, Columbia University, New York), YAT1 (MATa zds1::TRP1) (9), YMM221 (MATa sc7::zds1::TRP1), YMM222 (MATa sc7), YMM180 (MATa swe1::HIS3::SWE1-9xMyc CLN2-3xHA), YMM187 (MATa zds1::TRP1 swe1::HIS3::SWE1-9xMyc CLN2-3xHA).

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Abbreviations: AdoMet, S-adenosyl-L-methionine; AdoHcy, S-adenosyl-L-homocysteine; YPD, yeast extract/peptone/dextrose; HA, hemagglutinin; FACS, fluorescence-activated cell sorter; PL, propidium iodide.

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3xHA), YMM223 (MATa zds1::TRPI sc7 swel::HIS3::SWE1-9xMyc CLN2-3xHA), YMM224 (MATa sc7 swel::HIS3::SWE1-9xMyc CLN2-3xHA), CC850-2B (MATa met4::TRPI met30::URA3) (gift from D. Thomas, National Centre de la Recherche Scientifique, Paris), YMM225 (MATa zds1::TRPI met4::TRPI), and YMM227 (MATa vps33::kanMX4). The vps33 strain was constructed by gene replacement. Genomic DNA was isolated from the vps33::kanMX4 strain of BY4741 background (Invitrogen). The VPS33 locus was amplified by PCR using primers 5'-TACAGAAGAGTCTGGAGTCGAT-3' and 5'-TGTTCCA-TTGTGCTCCTAGTT-3'. The amplified fragment was used to transform the W303 strain. B medium, specifically designed to study sulfur metabolism, contained only essential amino acids, vitamins, and mineral salts, but no sulfur compounds except 0.5 mM L-cysteine (13). O medium, used for the accumulation of AdoMet, contains a high level of methionine (0.15%) (14). Other media used in the present study were as described (9). The strain with a chromosomally integrated gene for the construction of Cln2p with a 3xHA epitope at its C terminus was prepared as follows: the chromosomal CLN2 gene was replaced with a gene encoding Cln2p with a triple hemagglutinin (HA) epitope tag at its C terminus, using plasmid pMT290 (gift from T. Kishi, National Institute of Genetics, Shizuoka, Japan; M. Tyers, Samuel Lunenfeld Research Institute, Toronto; and B. Futcher, Stony Brook University, Stony Brook, NY).

Construction of Plasmids. The plasmid Ylp5-SAHI was constructed to establish allelism between SCZ7 and SAHI. The plasmids pUC119-SAHI, Ycp50-SAHI, and Ylp5-SAHI were constructed as follows. The entire ORF of the plasmids pUC119-SAH1, YCp50-SAH1, and Ylp5-SAH1 were constructed to establish allelism between SCZ7 and SAHI. Comparison of Scz7p and YER043c sequences suggested that it encodes a protein conserved among eukaryotes. Comparison of Scz7p and YER043c sequences revealed the presence of three amino acid substitutions, i.e., I291V, T279V, and I291V. Of these substitutions, the site-directed mutagenesis experiment revealed that the T279I mutation at a conserved residue was responsible for the observed phenotypes (data not shown). Hereafter, we shall refer to the SCZ7 gene as SAHI and the scz7 mutation as sahi-1.

Site-Directed Mutagenesis. To identify the mutation site responsible for the phenotype of sahi-1 strain by site-directed mutagenesis, we constructed three plasmids, each containing an SAHI gene as an allele of SAHI gene that encodes S-adenosyl homocysteine hydrolase. The growth defect and polarized bud formation of a zds1 Δ strain, as an allele of SAHI gene that encodes S-adenosyl homocysteine hydrolase. The growth defect and polarized bud formation at a conserved residue was responsible for the observed phenotype was recovered. The plasmid contained the YER043c ORF encoding a polypeptide similar to the human enzyme involved in the hydrolysis of AdoHcy to adenosine and homocysteine (12) and was designated as the SAHI gene. Site-directed mutagenesis was confirmed by DNA sequencing. The low-copy plasmid Ycp50-SAHI (wild type, I274V, T279I, or I291V) was generated as follows: the pUC119-SAHI containing SAHI (wild type) or SAHI (I274V, T279I, or I291V) was ligated into the HindIII-SphI site of Ycp50 and used for complementation analysis of the sahl-1 phenotypes.

RNA Isolation and Northern Blot Analysis. The SWE1, CLN2, and ACT1 probes were generated by random-primed labeling of a 0.7-kb BglII fragment of SWE1, a 1.3-kb NcoI-XhoI fragment of CLN2, and a 1.1-kb XhoI-KpnI fragment of ACT1, respectively, with [α-32P]dCTP by use of a multiprime DNA labeling kit (Amersham Pharmacia Biosciences).

Western Blot Analysis. The cells were washed and harvested by centrifugation. Pellets were resuspended in 50 μl of 2x sample buffer and lysed by vortexing for 15 min with an equal volume of glass beads at 4°C. After removal of the glass beads, the lysates were boiled for 5 min. Proteins were resolved by SDS/PAGE on a 7.5% or 12% gel and analyzed by immunoblotting. For detection of the HA-tagged proteins, Myc-tagged proteins, and Cdc28p, monoclonal antibodies 12CA5 (Babco, Richmond, CA) against the HA epitope, 9E10 (Babco) against the Myc epitope, and anti-PSTAIRE (Santa Cruz Biotechnology), respectively, were used.

Extraction and Analysis of AdoMet and AdoHcy. Extraction of AdoMet and AdoHcy was carried out as described (14). Briefly, cells growing in log-phase (OD600 = 0.2–0.3) were collected by centrifugation, washed, and then extracted with 1 ml of 10% perchloric acid for 1 h at room temperature. The resultant supernatant was diluted with MilliQ-grade water, and the samples were filtered for capillary electrophoresis. Determination of AdoMet and AdoHcy was performed by capillary electrophoresis by using a Waters Capillary Ion Analyzer with an Accusep fused silica gel column (60-cm total length and 75-μm i.d.) (15). The retention times of AdoMet and AdoHcy were 8.1 and 12 min, respectively. The AdoMet and AdoHcy content were expressed as nmol per mg dry weight of cells.

All other methods used were as described (9).

Results

Identification of scz7 Mutation, a Suppressor of Ca2+ Sensitivity of zds1 Δ Strain, as an Allele of SAHI Gene That Encodes S-Adenosyl Homocysteine Hydrolase. The growth defect and polarized bud growth exhibited by zds1 Δ cells on solid medium containing CaCl2 (100 to 300 mM) were suppressed by the scz7 mutation (Fig. 2 A and B). Fluorescence-activated cell sorter (FACS) analysis showed that the G2 cell-cycle delay was also suppressed by the mutation (Fig. 2C). In addition to the suppressor phenotype, the scz7 mutant exhibited, on both zds1 Δ and ZDS1 backgrounds, a slow growth phenotype at all temperatures examined (14°–37°C), with more severe retardation of growth at extreme temperatures (Fig. 2A).

For characterization of the scz7 mutation, a centromeric genomic library was introduced into the scz7 strain, and one plasmid that complemented the slow growth phenotype was recovered. The plasmid contained the YER043c ORF encoding a polypeptide similar to the human enzyme involved in the hydrolysis of AdoHcy to adenosine and homocysteine (12) and was designated as the SAHI gene. Linkage analysis showed that the scz7 mutation resided in this SAHI gene (data not shown). According to the whole genome approach for S. cerevisiae, SAHI is considered to be an essential gene (16). A blast search revealed that the deduced amino acid sequences of the SAHI gene showed similarity to those of Schizosaccharomyces pombe SPBC2D18.12c, Drosophila melanogaster Aheyl3, and human AHY, suggesting that it encodes a protein conserved among eukaryotes. Comparison of Scz7p and YER043c sequences revealed the presence of three amino acid substitutions, i.e., I274V, T279V, and I291V. Of these substitutions, the site-directed mutagenesis experiment revealed that the T279I mutation at a conserved residue was responsible for the observed phenotypes (data not shown). Hereafter, we shall refer to the SCZ7 gene as SAHI and the scz7 mutation as sahi-1.

Characterization of the sahi-1 Mutation as a Suppressor of Ca2+-Sensitive Phenotypes of zds1 Δ Strain. The effect of Ca2+ on the growth of zds1 Δ strain was shown earlier to be exerted by the activation of Swelp, a negative regulatory kinase of the Cdc28p/Cib2p complex (9–11, 17, 18). Under the conditions, the level of Cdc28p and G1 cyclin, was also found to be elevated (unpublished result). For further characterization of the sahi-1 mutation, we examined the effect of this mutation on the levels of SWE1 and

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CLN2 mRNA by Northern blot analysis. The mRNA levels of the zds1Δ strain were substantially decreased by the sah1-1 mutation, even in the presence of CaCl2 (compare zds1Δ and zds1Δ sah1-1 in Fig. 3A). We next examined the levels of Swe1p and Cln2p by Western blot analysis of the cells with chromosomally integrated constructs for Myc-tagged Swe1p and HA-tagged Cln2p. Reflecting the low levels of the mRNA, the Swe1p and Cln2p levels were low in the sah1-1 strain in the presence and absence of CaCl2 (compare zds1Δ and zds1Δ sah1-1 in Fig. 3B). The inability to elevate the Swe1p and Cln2p levels in the presence of CaCl2 provided the basis for the suppression of the Ca2⁺-related phenotypes of zds1Δ strain by the sah1-1 mutation. Basically similar effects of the sah1-1 mutation were seen on the ZDS1 background (compare wild-type and sah1-1 in Fig. 3 A and B). We further determined the SWE1 and CLN2 mRNA levels during the cell cycle, by using the α-factor-treated sah1-1 and wild-type cells (Fig. 3C). In contrast to the wild-type cells, the SWE1 and CLN2 mRNA levels in the sah1-1 cells were undetectably low, showing that the sah1-1 mutation caused a defect in the regulation of SWE1 and CLN2 genes at the transcriptional level and led to a cell-cycle delay in G1 (Fig. 3C). We further examined the effect of the sah1-1 mutation on the mRNA stability of SWE1 and CLN2 by Northern blot analysis. However, convincing results were not available due to the very low initial levels of SWE1 and CLN2 in sah1-1 cells, even when these genes were overexpressed from own promoter on 2μ plasmid (data not shown). Thus, the sah1-1 mutation seemed to lead to a defect in the elevation of the SWE1 and CLN2 mRNA levels. Because basically a similar effect of the sah1-1 mutation on the SWE1 and CLN2 expression was observed on both zds1Δ and wild-type (ZDS1) backgrounds, we used the wild-type background in most of the subsequent experiments.

Defect of AdoHcy Hydrolase in the sah1-1 Cells. If Sah1p is indeed involved in the hydrolysis of AdoHcy, and the sah1-1 mutant is impaired in its activity, then AdoHcy would be accumulated in the mutant cells (Fig. 1). So we determined the cellular content of AdoHcy and its immediate precursor, AdoMet, in wild-type and the mutant strains. The amounts of AdoMet and AdoHcy in the cell extracts were determined by capillary electrophoresis. Although the cellular content of AdoHcy in the wild-type strain was undetectably low, its content in the zcs7 strain was elevated to 0.31 nmol per mg of dry cells (Table 1). Surprisingly, concomitant with the increased amount of AdoHcy, the amount of AdoMet was also elevated in the mutant (1.95 nmol per mg...
of dry cells). In the medium supplemented with a high level of methionine (0.15%; O-medium), the cellular contents of AdoHcy and AdoMet in wild-type cells were elevated to measurable levels (0.16 and 0.36 nmol per mg of dry cells, respectively). In sah1-1 cells, the contents of AdoHcy (1.35 nmol per mg of dry cells) and AdoMet (13.40 nmol per mg of dry cells) were increased to 8.4- and 37.2-fold, respectively, of wild-type cells (Table 1). These results indicate that AdoHcy hydrolase was defective in the sah1-1 strain and that the cellular accumulation of AdoHcy in the mutant was accompanied by an increase in the content of AdoMet by unknown mechanism.

To examine whether the growth defect in the sah1-1 strain was caused by the accumulation of AdoHcy and/or AdoMet, we compared the effect of exogenous AdoMet, AdoHcy, and methionine on the growth of sah1-1 and wild-type strains. Because the effect of these supplements on cell growth was marginal in YPD (yeast extract/peptone/dextrose) medium, we used a specific synthetic medium (B medium) containing a limited amount of sulfur atoms (13). As shown in Fig. 4, the growth of both wild-type and sah1-1 strains was severely inhibited by the exogenous AdoHcy. In contrast, the exogenous l-methionine and AdoMet significantly improved the growth of sah1-1 strain. From these results, the poor growth of the sah1-1 strain would seem to be due to the cellular accumulation of AdoHcy, and the concomitant accumulation of AdoMet in the mutant cells seemed to improve the growth of the mutant and led to the suppression of the Ca2+ sensitivity.

### Table 1. Cellular contents of AdoMet and AdoHcy in wild-type and sah1-1 cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>YPD</th>
<th>O medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>sah1-1</td>
<td>Wild type</td>
</tr>
<tr>
<td>AdoMet, nmol/mg cells</td>
<td>ND</td>
<td>1.95</td>
</tr>
<tr>
<td>AdoHcy, nmol/mg cells</td>
<td>ND</td>
<td>0.31</td>
</tr>
<tr>
<td>AdoMet/AdoHcy</td>
<td>ND</td>
<td>6.3</td>
</tr>
</tbody>
</table>

The content of AdoMet and AdoHcy were measured using early log-phase growing cells (OD600 of 0.2–0.3) of wild-type (DHT22-1b) and sah1-1 (YMM222) strains in YPD or O medium. The values are means from two independent experiments. ND, not detected.

**Elevation of AdoMet Leads to a Delay of the Cell Cycle in G1 by the Down-Regulation of SWE1 and CLN2 Expression.** Because the growth inhibition of the zds1Δ strain by Ca2+ is mediated by the elevation of SWE1 and CLN2 gene expression, and the expression levels of these genes were markedly reduced in the sah1-1 strain, we presumed that the cellular accumulation of AdoMet and/or AdoHcy may lead to the decreased expression of these genes. To test this possibility, we first examined the effects of AdoMet and AdoHcy on the SWE1 and CLN2 mRNA levels in wild-type cells. As expected, the exogenous AdoMet or AdoHcy led to a sharp decrease in the levels of SWE1 and CLN2 mRNA in the wild-type cells (Fig. 5A). Consistent with the low levels of the mRNAs, Swe1p and Cln2p levels were decreased by the exogenous AdoMet or AdoHcy, but not by l-methionine (Fig. 5B). These results suggest that the cellular accumulation of AdoMet and AdoHcy led to the inhibition of SWE1 and CLN2 expression. Furthermore, FACS analysis revealed that the exogenous AdoMet led to a cell-cycle delay in G1 (Fig. 5C). It should be noted, however, that the AdoMet-induced G1 delay was only transient (~3 h, Fig. 5C) and that the cells resumed growth during further incubation (data not shown). It was earlier shown that hyperaccumulation of AdoMet caused a growth defect in the strain deleted for the VPS33 gene encoding a component essential for vacuolar morphogenesis (19, 20). We, therefore, examined the effect of AdoMet in the vps33Δ strain and found that exogenous AdoMet led the cells to G1 cell-cycle delay in YPD medium (Fig. 5D). A similar result was obtained in B medium (data not shown). Further, the growth of the vps33 strain was inhibited by exogenous AdoMet (data not shown). In contrast to the G1-specific effect of AdoMet, the effect of AdoHcy was unrelated to the cell-cycle phase (Fig. 5C). These results suggest that, although AdoMet and AdoHcy similarly down-regulated SWE1 and CLN2 expression, the effects of these compounds on cell-cycle regulation were distinct (see Discussion).

**Defect of SAH1 Gene Led to G1 Cell-Cycle Arrest.** We next examined whether the sah1-1 mutant exhibited a cell cycle-specific arrest
phenotype at the restrictive temperature (37°C). It was noted that unbudded G1 cells were accumulated on shift of the sah1-1 strain to 37°C (data not shown). So we examined the arrest point of sah1-1 cells in synchronized cell cultures. The mutant cells were synchronized in the G1-phase by a-factor treatment and in the S-phase by hydroxyurea treatment; and the treated cells were then released into fresh YPD medium at 37°C, the restrictive temperature of the mutant. FACS analysis demonstrated that the S-phase-arrested sah1-1 cells proceeded through the cell cycle normally after their release (Fig. 6). In a converse experiment, the growth of the sah1-1 cells was first arrested at 37°C, released into the medium containing a-factor and further incubated at 25°C. As expected, the cells did not progress into S-phase (data not shown). These results enforced the conclusion that the arrest point in the sah1-1 mutant was at or very close to the phenome arrest point in G1-phase, before S-phase.

Exogenous AdoMet Suppresses the Ca2+-Phenotypes of zds1Δ Strain in a Manner Independent of Met4p. We presumed that the exogenous AdoMet suppressed the Ca2+-induced growth arrest of zds1Δ cells through the down-regulation of SWE1 and CLN2 expression. As expected, the growth inhibition of zds1Δ cells by 300 mM CaCl2 was partially suppressed by the exogenous AdoMet (Fig. 7).

The degradation of Met4p is mediated by the ubiquitin ligase SCF^{Met30}, which is activated in response to the elevation of intracellular AdoMet (21). If the degradation of Met4p is required for suppressing the Ca2+ phenotypes of the zds1Δ strain, the Ca2+-sensitivity would be suppressed by the deletion of the MET4 gene. However, zds1Δ met4Δ cells were even more sensitive to Ca2+ than the zds1Δ cells (Fig. 7). Furthermore, AdoMet could partially suppress the Ca2+ sensitivity of zds1Δ met4Δ (Fig. 7). These results indicate the presence of a Met4p-independent mechanism in the down-regulation of SWE1 and CLN2 by AdoMet.

Discussion

The scz7/sah1-1 mutation, an allele of the SAH1 gene, was isolated as a suppressor of the Ca2+ sensitivity of the zds1Δ strain. The Ca2+-sensitive phenotypes, namely, the growth defect, the G2 cell-cycle delay, and the polarized bud growth of the zds1Δ cells, were all caused by the elevation of Swelp. Thus, all these defects can be suppressed by the deletion of the SWE1 gene (9). Moreover, we found that the CLN2 mRNA and Cln2p were also elevated by Ca2+ (Fig. 3 A and B and unpublished results).

In the sah1-1 mutant, the increase in the cellular content of AdoHcy and AdoMet was accompanied by the decrease in the levels of the Swelp and Cln2p. Thus, the suppressor effect of the sah1-1 mutation seems to be attributable to the defect of the elevation of the Swelp and Cln2p levels in response to Ca2+. Consistent with this interpretation, the exogenous AdoHcy and AdoMet led to a G1 cell-cycle delay in the strain lacking the vacuoles, the delay observed in the wild-type strain was only transient. The vacuole is important in the maintenance of cellular AdoMet homeostasis to protect cells from the growth inhibitory effect of AdoMet (19). Although exogenous AdoHcy and AdoMet similarly down-regulated the Swelp and Cln2p expression in the wild-type strain, the effects of these compounds on the cell cycle were distinct. Because AdoHcy is a potent competitive inhibitor of various AdoMet-dependent methyltransferases (22), the accumulation of AdoHcy is harmful for cell growth (23). Thus, the cell-cycle nonspecific effect of AdoHcy on cell growth may be explained by the inhibition of various AdoMet-dependent methyltransferases in multiple essential processes needed for cell growth.

In the sah1-1 strain, the cellular accumulation of the AdoHcy, the substrate of AdoHcy hydrolase, was accompanied by the remarkable accumulation of AdoMet. It is known that the growth of cystathionine β-synthase mutant became inhibited when the intracellular AdoMet/AdoHcy ratio dropped below 1.5 (23). In sah1-1 mutant cells, the AdoMet/AdoHcy ratio in the cells grown in YPD medium was 6.3. The elevation of the cellular AdoMet level in sah1-1 cells would seem to be the result of up-regulation of the AdoMet production from methionine by unknown mechanism to cope with the critical conditions due to the detrimental accumulation of AdoHcy.

The results of an earlier study indicated that uncontrolled expression of Met4p negatively regulated the SWE1 gene and further incubated at 25°C for 3 days.
gene network have a harmful effect on the passage through Start (3). It is possible that the effect of the increase in the cellular AdoMet level in sahl-1 strain is mediated by Met4p, leading to the G1 delay. If so, the cell cycle delay should be abolished by the deletion of the MET4 gene. However, the growth of the sahl-1 strain was not suppressed by the MET4 deletion, but the growth was aggravated by the met4Δ mutation, exhibiting cell-cycle arrest in G1 (data not shown). Similarly, the deletion of MET4 failed to suppress the Ca2+ sensitivity of the zds1Δ strain, and the exogenous AdoMet suppressed the Ca2+ sensitivity of zds1Δ met4Δ cells (Fig. 7). It is thus unlikely that Met4p is involved in the AdoMet-induced cell-cycle regulation. However, it is also possible that Met4p and/or Met30p have additional functions in growth regulation, besides the control of the G1-S transition.

The mechanisms by which AdoMet down-regulates the SWEI and CLN2 gene expression are still unknown. AdoMet may inhibit the transcription of SWE1 and CLN2 genes through the regulation of the transcription factors SBF (Swi4p-Swi6p) and MBF (Mbp1p-Swi6p), which mediate cell cycle-specific transcription in late G1 (24–26). However, the deletion of SWI4 or SWI6 gene led to a delay in G2, but not in G1, suggesting that the effect of AdoMet is unlikely to be mediated by the SBF and MBF transcription factors (our unpublished result). Alternatively, it is possible that AdoMet inhibits the activity of the general transcription machinery. It is also possible that AdoMet is involved in the regulation of the rate of SWE1 and CLN2 transcription and/or the rate of their mRNA degradation. Consistent with these possibilities, we found that SWE1 and CLN2 mRNA levels were very low in the sahl-1 cells, even when these genes were overexpressed from their own promoter on 2μ plasmid (data not shown).

Under starvation conditions, yeast cells are arrested in the G1-phase (27). Several pathways respond by causing changes in cellular metabolism. For example, the TOR2 pathway and cAMP pathway modulate several nutritional signaling pathways (28–31). It is possible that some of the changes in intermediate levels may modulate the cell cycle. As seen in this study, a high level of intracellular AdoMet may serve as a signal for the arrest or delay in G1.

AdoHcy has received much attention as a possible endogenous pathogenic compound (23). In humans, elevated AdoHcy is associated with vascular disease (32). The sahl-1 strain may be useful as a model organism to further our knowledge of the consequences of the cellular accumulation of AdoHcy. Extensive genomic profiling and proteomic analysis will help to identify novel targets of AdoMet and/or AdoHcy. It is also of interest to investigate whether AdoMet affect Ca2+ signaling pathways. Our present findings of the newly recognized effects of AdoMet and AdoHcy will provide additional insights into the regulation of the cell cycle in both yeasts and higher organisms, and into the central role played by AdoMet in cellular metabolism.

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