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**Outline of Research Result**

# Analysis of the Effect of *S*-adenosylmethionine on Cell Growth in Yeast

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## Research aims

*S*-adenosyl-L-methionine (AdoMet) is a high-energy sulfonium compound used in most of the numerous biological methylation reactions. An alteration in the availability of AdoMet has a profound effect on cell growth. In a previous study, we isolated a mutant allele of *Saccharomyces cerevisiae* gene *SAH1* encoding *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase, as a mutation that suppressed the $Ca^{2+}$-sensitive phenotypes of the *zds1Δ* strain, such as the $Ca^{2+}$-induced, $G_2$ cell-cycle arrest and polarized bud growth[1,2]. Interestingly, the mutation (*sah1-1*) led the cells to accumulate AdoMet besides AdoHcy, the substrate of this hydrolase. A case of AdoHcy hydrolase deficiency in humans was also reported to cause the patient's leukocyte DNA to be hyper-methylated relative to the control level due to extreme levels of AdoHcy[3]. Similar to that in yeast, the defect in AdoHcy hydrolase in humans causes simultaneous elevation of AdoHcy and AdoMet. Thus the *sah1-1* mutant is a useful model to deepen our knowledge of the consequences of cellular accumulation of AdoHcy and AdoMet. The aim of this study was to investigate the phenotypes of the *sah1-1* mutant and elucidate the role of AdoMet and AdoHcy in yeast cell growth.

## Methods

*Saccharomyces cerevisiae* (wild-type, *sah1-1* or *vps33Δ*) cells were grown in rich medium (YPD). Unless otherwise indicated, all yeast strains were grown routinely at 25°C. Extraction of AdoMet and AdoHcy was carried out as described previously[1]. Determination of AdoMet and AdoHcy was performed by capillary electrophoresis using a Waters Capillary Ion Analyzer (Milford, MA, USA) with an Accusep fused silica gel column (60-cm total length and 75-$\mu$m ID)[1]. RNA isolation and DNA microarray profiling experiments were performed as described earlier[4].

## Results

In previous work, we showed that the cellular content of AdoHcy and AdoMet in the *sah1-1* strain was elevated in YPD medium at 25°C, a permissive temperature for *sah1* mutants[1]. It was earlier shown that hyper-accumulation of AdoMet caused a growth defect in a strain in which the *VPS33* gene encoding a non-essential Class C vacuolar sorting protein had been deleted. Therefore, we constructed a *sah1-1 vps33Δ* strain, and examined its growth. As expected, the *sah1-1 vps33Δ* strain was not viable, suggesting that intracellular AdoHcy and AdoMet had accumulated in vacuoles and that high levels of AdoHcy and AdoMet were harmful to yeast cell growth.

We have shown that the *sah1-1* mutant exhibited a slow growth phenotype at all temperatures examined (14～37°C), with more severe retardation of growth at extreme temperatures. We expected that the intracellular content of AdoHcy and AdoMet in the *sah1-1* strain would be elevated at a high temperature. Therefore we determined the cellular content of AdoHcy and AdoMet in the *sah1-1* strain in YPD medium at 37°C, a non-permissive temperature for *sah1* mutants. The amounts of AdoHcy and AdoMet in the cell extracts were determined by capillary electrophoresis. The cellular contents of AdoHcy and AdoMet in the *sah1-1* strain in YPD medium were about 2.5- and 6-fold, respectively, higher at 37°C than at 25°C. In the *sah1-1* strain, the cellular accumulation of the AdoHcy was accompanied by the remarkable accumulation of AdoMet. It is known that the growth of a cystathionine $\beta$-synthase mutant becomes inhibited when the intracellular AdoMet/AdoHcy ratio drops below 1.5[5]. We previously showed that the AdoMet/AdoHcy ratio in the *sah1-1* cells grown in YPD medium at 25°C was 6.3[1]. At 37°C, this ratio was 13.4. The elevation of the AdoMet/AdoHcy ratio at the high temperature in *sah1-1* cells would seem to be the result of up-regulation of the AdoMet production from methionine by some unknown mechanism to cope with the critical conditions due to the detrimental accumulation of AdoHcy. Further, we found that unbudded enlarged $G_1$ cells accumulated upon shifting of the *sah1-1* strain to 37°C. To validate the above result, we assayed the distribution of the cells throughout the cell cycle via fluorescence-activated cell sorting (FACS), measuring the numbers of cells with 1 (1C) and 2 (2C) copies of the chromosomes. The FACS data confirmed that *sah1-1* cells in the YPD medium at 37°C were delayed in the $G_1$ phase.

To test if elevation of the AdoHcy and AdoMet contents of the *sah1-1* cells affected gene transcription, we conducted DNA microarray analysis. Inspection of the full data set revealed several characteristics of the *sah1-1*-dependent gene. We identified genes whose expression was increased

more than 2-fold in the *sah1-1* cells at 25°C. In total, 64 genes were induced more than 2-fold; and they were those involved in lipid biosynthesis, phosphate/polyphosphate biosynthesis, and methionine biosynthesis. Interestingly, *SAH1* was also up-regulated at the transcriptional level. Next, we carried out the DNA microarray analysis on the *sah1-1* cells at 37°C. Unfortunately, we could not detect any signal for some unknown reason.

## Conclusion

The *sah1-1* mutation, an allele of the *SAH1* gene, was characterized in this study. In the *sah1-1* strain, the cellular accumulation of the AdoHcy, the substrate of AdoHcy hydrolase, was accompanied by the remarkable accumulation of AdoMet. This phenotype was enhanced by a shift to a higher temperature (37°C). At the higher temperature, the *sah1-1* cells were delayed in the $G_1$ phase of the cell cycle. DNA microarray analysis demonstrated that accumulation of AdoHcy and AdoMet in the *sah1-1* cells was due to the up-regulation of the mRNA of enzymes involved in methionine biosynthesis. Further analyses are necessary to expand our knowledge of the consequences of the cellular accumulation of AdoHcy and AdoMet.

## References


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