Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 343 (2006) 203–207
BBRC
www.elsevier.com/locate/ybbrc

Methylthioadenosine and polyamine biosynthesis in a *Saccharomyces cerevisiae meu1Δ* mutant

Manas K. Chattopadhyay, Celia White Tabor, Herbert Tabor *

Laboratory of Biochemistry and Genetics, Building 8, Room 223, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Received 22 February 2006
Available online 3 March 2006

Abstract

As part of our studies on polyamine biosynthesis in yeast, the metabolism of methylthioadenosine was studied in a mutant that lacks methylthioadenosine phosphorylase (*meu1Δ*). The nucleoside accumulates in this mutant and is mainly excreted into the culture medium. Intracellular accumulation of the nucleoside is enough to account for the inhibition of spermidine synthase and thus to indirectly regulate the polyamine content of the *meu1Δ* cells. By comparing the results with this mutant with a *meu1Δ spe2Δ* mutant that cannot synthesize spermidine or spermine, we showed that >98% of methylthioadenosine is produced as a byproduct of polyamine synthesis (i.e., from decarboxylated *S*-adenosylmethionine). In contrast, in $MEU1^{+}$ $SPE2^{+}$ cells methylthioadenosine does not accumulate and is metabolized through the methionine salvage pathway. Using a *met15Δ* mutant we show that this pathway (i.e., involving polyamine biosynthesis and methylthioadenosine metabolism) is a significant factor in the metabolism of methionine, accounting for 15% of the added methionine.
Published by Elsevier Inc.

Keywords: Methionine; Methylthioadenosine; Methionine salvage pathway; Polyamines; Spermidine; Spermine; Yeast

Methylthioadenosine is formed from decarboxylated *S*-adenosylmethionine during the biosynthesis of spermidine and spermine [1–3], and is then converted back to methionine by the "methionine salvage" pathway [4–9]. The first step in the methionine salvage pathway is carried out by 5′-methylthioadenosine/*S*-adenosylhomocysteine nucleosidase in some bacteria and plants, and by methylthioadenosine phosphorylase in mammals ([10] and references therein) and yeast [7,8]. The biosynthesis, metabolism, and physiological role of methylthioadenosine have been of particular interest because of its regulatory effect on polyamine biosynthesis [11–13] and because a number of tumor cell lines [14–17] lack the first enzyme in the methionine salvage pathway (methylthioadenosine phosphorylase).

As part of our overall interest on polyamine biosynthesis in yeast, we felt that it was important to test whether the biosynthesis of polyamines is the only pathway for the synthesis of methylthioadenosine in yeast, since other possible pathways have been reported in several in vitro studies [18–20]. In addition, we wanted to carry out quantitative studies to evaluate whether this pathway is a significant component in the overall utilization of methionine by yeast cells.

To answer these questions, we have taken advantage of the availability of a mutant of *Saccharomyces cerevisiae* (*meu1Δ*) that is unable to metabolize methylthioadenosine [21,22]. Using this mutant we have shown that essentially all of the methylthioadenosine formed in yeast is a product of polyamine biosynthesis and that this pathway represents 10–15% of the methionine used by the cell. In addition, we have shown that the amount of methylthioadenosine accumulated in the *meu1Δ* mutant is sufficient to account for the inhibition of spermidine biosynthesis reported in our recent paper on the effect of the *meu1Δ* mutation on polyamine regulation in *S. cerevisiae* [23].

* Corresponding author. Fax: +1 301 402 0240.
E-mail address: tabor@helix.nih.gov (H. Tabor).

0006-291X/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.bbrc.2006.02.144

Materials and methods

Yeast strains and media. Yeast strains Y607 (*MATα his3 leu2 lys2 ura3 Δmeu1*::KANMX) and Y610 (*MATα his3 leu2 lys2 ura3 MEU1*$^{+}$) were obtained from the yeast deletion bank. Y614 (*MATα his3 leu2 ura3 Δspe2*::*LEU2 Δmeu1*::KANMX) was obtained by crossing Y607 with the *spe2Δ* mutant (Y343) previously described [24]. The strains were maintained on YPAD plates (1% yeast extract, 2% Bactopeptone, 2% dextrose, and 2% agar). Cultures were inoculated from YPAD plates into SD medium (6.7 g yeast nitrogen base, 20 g dextrose, and necessary supplements per liter) and harvested in the logarithmic phase of growth (0.8–1.0 OD_{600}). The cell pellets were extracted with 5 volumes of 10% perchloric acid.

Estimation of methylthioadenosine and polyamines. Methylthioadenosine was quantitated by reverse-phase chromatography on a dC18 column as described by Kamatani and Carson [11]. One hundred to 200 μl of the above perchloric acid extract or 200 μl of the spent culture medium was injected into a dC18 column (4.6 × 150 mm, Atlantis column, Waters, 3 μm particle size, part No. 186001342). The elution buffer (8% acetonitrile, 10 mM potassium phosphate, pH 6.0) was run at 0.8 ml/min, and the UV absorbance of the eluate was measured at 254 nm. Methylthioadenosine was eluted at 32.5 min. For further characterization of the eluted material, fractions from 31–34 min of the HPLC chromatography were collected after a larger injection (2 ml), dried, and the HPLC was repeated. This material was then characterized by electrospray ionization mass spectrometry (LCT Premier time-of-flight (TOF) mass spectrometer, Waters) and UV absorbance spectra. The polyamine content of these extracts was determined by HPLC on a cation exchanger as previously described [25].

For the experiments on growth in limiting methionine, strain Y606 (*MAT*a *his3 leu2 met15 ura3 meu1Δ*::KANMX) was obtained from the yeast deletion bank; this mutant is blocked in the utilization of sulfur for the synthesis of methionine and cysteine, Y606 inoculated in the same SD medium as above, but with the addition of 5–10 μM (final concentration) of L-methionine. Cultures were harvested after growth stopped (OD_{600} = 0.3–0.5). Aliquots of the spent medium were then assayed for methylthioadenosine as above. For [^{35}S]methionine incorporation, Y606 cultures were grown in 10 μM unlabeled L-methionine and 0.25 μCi of L-[^{35}S]methionine per milliliter of culture medium (GE Amersham, 1000 Ci/mmol, SJ1015) until the growth stopped (0.5 OD_{600}). The cells were harvested and treated with 10% perchloric acid as above. Aliquots of this extract and of the spent medium were fractionated on the dC18 column. The peak fractions (31–33 min) were collected and aliquots were counted in a scintillation counter. The perchloric acid-insoluble pellet was washed and dissolved in 0.1 M Tris–HCl (pH 9.5), prior to counting.

Results

Methylthioadenosine accumulates in meu1Δ SPE2^{+} *cultures and is absent in meu1Δ spe2Δ cultures*

An *meu1Δ* culture (Y607) was grown to an optical density of 0.9. Analysis of the cell extracts and of the culture medium showed that these cultures accumulated methylthioadenosine, and that most of the accumulated material was excreted into the medium (Figs. 1 and 2). The elution profile showed a clear peak of methylthioadenosine, which is well separated from other components present in either the culture medium or in the cell extract (Fig. 1). Even though most of the accumulated methylthioadenosine was excreted into the culture medium (1155 nmol in 385 ml of culture medium), about 2% of the total accumulation was found in cells (23 nmol in 754 mg of wet weight of cells or approximately 680 μl of cell volume) giving a calculated intracellular concentration of 3×10^{-5} M.

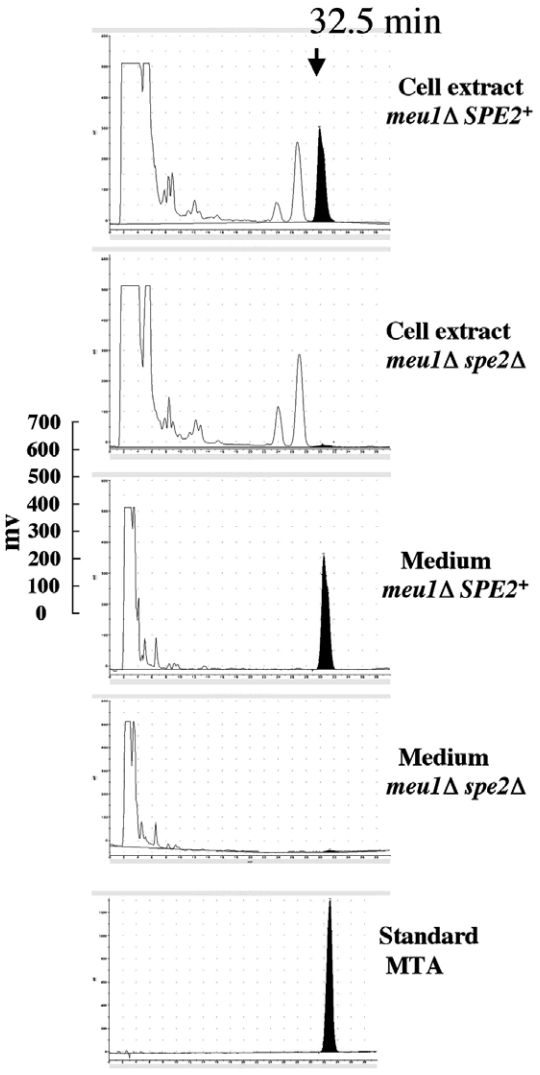

Fig. 1. Methylthioadenosine accumulates in *meu1ΔSPE2*$^{+}$ cultures and is absent in *meu1Δ spe2Δ* cultures. Cultures of Y607 (*meu1Δ SPE2*$^{+}$) and of Y614 (*meu1Δ spe2Δ*) were grown to an OD of 0.9. Cells were harvested and perchloric acid extracts of the cells were prepared as described in Materials and methods. Aliquots of the cell extract (100 μl) or of the spent medium (200 μl) were then assayed by HPLC dC18 chromatography as described in Materials and methods. These chromatograms are representatives of several assays. The arrow (32.5 min) indicates the peak of standard methylthioadenosine, and the scale is shown on the left side.

As opposed to the results with the *meu1Δ* cultures, only traces of methylthioadenosine were found in the cells or medium of *meu1Δ spe2Δ* (Fig. 1) or *MEU1*$^{+}$ *SPE2*$^{+}$ cultures (data not shown).

Methylthioadenosine was identified by its position on the HPLC chromatograph (32.5 min), by UV absorption spectrum (peak: 257 nm), by mass spectrometry (found 298.0978 Da; calculated for M + 1 298.0974 Da)[1], and by labeling with L-[^{35}S]methionine (see below).

[1] We thank Dr. John Lloyd, Proteomics and Mass Spectrometry Facility, NIDDK, for performing the mass spectrometric analysis.

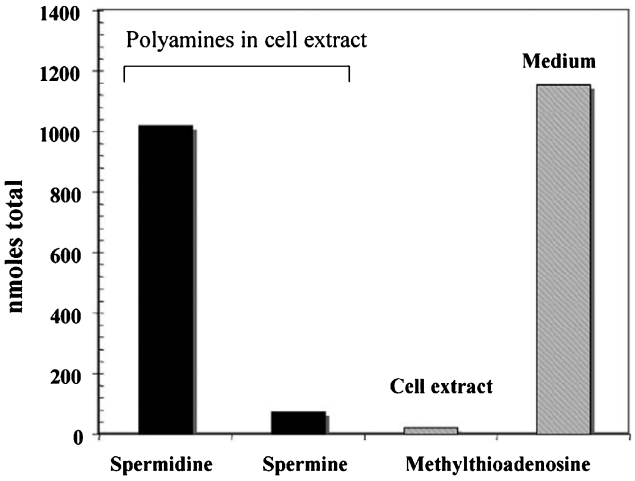

Fig. 2. Equivalence of polyamine biosynthesis and methylthioadenosine accumulation in Y607 (*meu1Δ SPE2*) cultures. Y607 cultures (385 ml) were grown to $OD_{600} = 0.9$ and harvested as described in Materials and methods. Aliquots of the perchloric acid extract of the cells were analyzed for polyamines and methylthioadenosine. Aliquots of the spent medium were analyzed for methylthioadenosine. The data are presented as the total amount of polyamines or of methylthioadenosine found in the culture (385 ml). The biosynthesis of 1020 nmol of spermidine would result in the production of 1020 nmol of methylthioadenosine; the biosynthesis of 75 nmol of spermine would result in the production of 150 nmol of methylthioadenosine.

Equivalence of polyamine biosynthesis and methylthioadenosine accumulation in Y607 (meu1Δ) cultures

The amount of methylthioadenosine found in the *meu1Δ SPE2*$^+$ cultures was equal, within the limit of our assays, to the amount of spermidine and spermine found in the cells (Fig. 2). These data indicate that essentially all of the accumulated methylthioadenosine was the product of polyamine biosynthesis. To establish this conclusion more definitively, we assayed the cells and medium from *spe2Δ meu1Δ* cultures (grown in 10^{-4} M spermidine). These cells cannot make decarboxylated adenosylmethionine, and thus cannot make spermidine or spermine. Only traces of methylthioadenosine were found in the medium of these cultures (Fig. 1).

Methionine salvage pathway accounts for 11–19% of the methionine utilized by S. cerevisiae

To test how much methionine passes through methylthioadenosine and the methionine salvage pathway, we used Y606 (*met15Δ meu1Δ*), which cannot synthesize methionine from inorganic sulfate and is blocked in the further metabolism of methylthioadenosine. We grew these cells on limiting methionine (5–10 μM), and after growth ceased we measured the amount of methylthioadenosine found in the spent medium and compared this value with the amount of methionine that had been added to the medium. As shown in Table 1, 11–19% of the methionine was converted to methylthioadenosine.

Table 1
Methionine salvage pathway accounts for 11–19% of the methionine utilized by *S. cerevisiae*

Methionine added to the medium (zero time)/ml	Final optical density at 600 nm	Methylthioadenosine spent medium/ml	% conversion, methionine to methylthioadenosine
5 nmol	0.3	0.8 nmol	16
8 nmol	0.5	1.5 nmol	19
10 nmol	0.5	1.1 nmol	11
4.6×10^5 CPM[a]		5×10^4 CPM	

[a] This experiment was performed in the presence of L-[^{35}S]methionine; 2.6×10^5CPM/ml were found in the cellular protein, presumably as methionine and cysteine i.e., 56% of total methionine added.

A comparable experiment was also performed with L-[^{35}S]methionine (Table 1, bottom row). Analysis of the culture medium after the growth stopped showed that 11% of the labeled methionine has been converted to labeled methylthioadenosine.

Discussion

Although enzymatic studies have shown that methylthioadenosine is formed stoichiometrically during the biosynthesis of spermidine and spermine [2], other enzymatic pathways have been described in vitro [18,19] and there have been little data on whether polyamine biosynthesis is the major source of methylthioadenosine in yeast in vivo. It has been known for a long time that methylthioadenosine could be formed by the non-enzymatic decomposition of *S*-adenosylmethionine [26]. In our current studies, we have shown by both analytic and genetic methods that at least 98% of the methylthioadenosine that accumulates in the *meu1Δ* mutant is formed via the polyamine biosynthetic pathway (Fig. 1) and is excreted into the culture medium where the concentration reaches 10^{-6} M (1155 nmol in 385 ml). We cannot interpret the trace amount found in the *spe2Δ meu1Δ* mutant at position 32.5 min of the HPLC chromatography as indicating another pathway since the amounts formed were too small to permit adequate identification.

These results with the yeast mutants are comparable to the findings of Kamatani and Carson [11] with cultures of leukemia cells that cannot metabolize methylthioadenosine because they lack methylthioadenosine phosphorylase. They showed that these cultures accumulate methylthioadenosine in the medium and that this accumulation is inhibited by methylglyoxal bis(guanylhydrazone), an inhibitor of *S*-adenosylmethionine decarboxylase.

Although it seemed obvious that the polyamine pathway represented a significant fraction of the methionine utilized by the cell, there have been very little quantitative data on this question. In our current work, we show that the polyamine biosynthetic pathway (and methylthioadenosine formation) is a significant factor in considering methionine metabolism and accounts for about 11–19% of the methionine used by a methionine auxotroph growing on limiting methionine (Table 1).

There has been particular interest in the physiological and regulatory effects of methylthioadenosine and of the *MEU1* gene since many tumor cells lack the *MEU1* gene, and there have been several studies indicating changes in polyamine levels in the absence of this gene in both mammalian and yeast cells [11,12,22,23]. In our recent paper [23] on the effect of the *meu1* mutation on polyamine levels in *S. cerevisiae*, we postulated an in vivo inhibition of spermidine synthase by accumulated methylthioadenosine to explain the changes observed in polyamine levels in the *meu1Δ* cells. A number of studies had shown such inhibition of spermidine synthase, spermine synthase, and other enzymes by methylthioadenosine in vitro [23,27–31]. In our current work we have found that, even though most of the accumulated methylthioadenosine is excreted into the medium, the intracellular level (3×10^{-5} M) is still sufficient to effect an inhibition of spermidine synthase in vivo and thereby affect the regulation of polyamine biosynthesis. In wild-type cells, methylthioadenosine does not accumulate and is rapidly metabolized further through the salvage pathway (data not shown). Hence, it seems unlikely that in the wild-type cells methylthioadenosine would exert a significant regulatory function.

It is of interest that the *MEU1* gene was first identified in yeast by Donoviel and Young [32] in their studies on a completely unrelated system; namely the involvement of this gene in the regulation of the *ADH2* gene. It is intriguing to speculate that methylthioadenosine may have some more general influence on enzyme regulation, possibly resulting from an alteration in polyamine biosynthesis.

In conclusion, the methionine salvage pathway involving polyamine biosynthesis and methylthioadenosine represents a small but significant pathway in recycling approximately 15% of methionine as well as producing adenine. In the *meu1Δ* cells, methylthioadenosine accumulates and is mostly excreted in the medium; the methylthioadenosine accumulated is equivalent to the amount of spermidine and spermine synthesized by the cell, indicating that polyamine metabolism in yeast is the major (more than 98%) contributor to methylthioadenosine production and that there is no substantial degradation of methylthioadenosine in the absence of methylthioadenosine phosphorylase.

Acknowledgment

This research was supported by the Intramural Research Program of the NIH (National Institute of Diabetes, Digestive, and Kidney Diseases).

References

[1] H. Tabor, S.M. Rosenthal, C.W. Tabor, The biosynthesis of spermidine and spermine from putrescine and methionine, J. Biol. Chem. 233 (1958) 907–914.

[2] A.E. Pegg, H.G. Williams-Ashman, On the role of *S*-adenosyl-L-methionine in the biosynthesis of spermidine by rat prostate, J. Biol. Chem. 244 (1969) 682–693.

[3] W.H. Bowman, C.W. Tabor, H. Tabor, Spermidine biosynthesis. Purification and properties of propylamine transferase from *Escherichia coli*, J. Biol. Chem. 248 (1973) 2480–2486.

[4] P.S.J. Backlund, C.P. Chang, R.A. Smith, Identification of 2-keto-4-methylthiobutyrate as an intermediate compound in methionine synthesis from 5′-methylthioadenosine, J. Biol. Chem. 257 (1982) 4196–4202.

[5] P.C. Trackman, R.H. Abeles, The metabolism of 1-phospho-5-methylthioribose, Biochem. Biophys. Res. Commun. 103 (1981) 1238–1244.

[6] P.C. Trackman, R.H. Abeles, Methionine synthesis from 5′-*S*-methylthioadenosine. Resolution of enzyme activities and identification of 1-phospho-5-*S* methylthioribulose, J. Biol. Chem. 258 (1983) 6717–6720.

[7] M.C. Cone, K. Marchitto, B. Zehfus, A.J. Ferro, Utilization by *Saccharomyces cerevisiae* of 5′-methylthioadenosine as a source of both purine and methionine, J. Bacteriol. 151 (1982) 510–515.

[8] K.S. Marchitto, A.J. Ferro, The metabolism of 5′-methylthioadenosine and 5-methylthioribose 1-phosphate in *Saccharomyces cerevisiae*, J. Gen. Microbiol. 131 (1985) 2153–2164.

[9] K.A. Cornell, R.W. Winter, P.A. Tower, M.K. Riscoe, Affinity purification of 5-methylthioribose kinase and 5-methylthioadenosine/*S*-adenosylhomocysteine nucleosidase from *Klebsiella pneumoniae* [corrected], Biochem. J. 317 (1996) 285–290.

[10] J.E. Lee, E.C. Settembre, K.A. Cornell, M.K. Riscoe, J.R. Sufrin, S.E. Ealick, P.L. Howell, Structural comparison of MTA phosphorylase and MTA/AdoHcy nucleosidase explains substrate preferences and identifies regions exploitable for inhibitor design, Biochemistry 43 (2004) 5159–5169.

[11] N. Kamatani, D.A. Carson, Abnormal regulation of methylthioadenosine and polyamine metabolism in methylthioadenosine phosphorylase-deficient human leukemic cell lines, Cancer Res. 40 (1980) 4178–4182.

[12] H. Yamanaka, M. Kubota, D.A. Carson, Synergistic inhibition of polyamine synthesis and growth by difluoromethylornithine plus methylthioadenosine in methylthioadenosine phosphorylase-deficient murine lymphoma cells, Cancer Res. 47 (1987) 1771–1774.

[13] M. Kubota, E.O. Kajander, D.A. Carson, Independent regulation of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase in methylthioadenosine phosphorylase-deficient malignant murine lymphoblasts, Cancer Res. 45 (1985) 3567–3572.

[14] J.I. Toohey, Methylthio group cleavage from methylthioadenosine. Description of an enzyme and its relationship to the methylthio requirement of certain cells in culture, Biochem. Biophys. Res. Commun. 78 (1977) 1273–1280.

[15] F. Della Ragione, G. Russo, A. Oliva, S. Mastropietro, A. Mancini, A. Borrelli, R.A. Casero, A. Iolascon, V. Zappia, 5′-Deoxy-5′-methylthioadenosine phosphorylase and p16INK4 deficiency in multiple tumor cell lines, Oncogene 10 (1995) 827–833.

[16] T. Nobori, K. Takabayashi, P. Tran, L. Orvis, A. Batova, A.L. Yu, D.A. Carson, Genomic cloning of methylthioadenosine phosphorylase: a purine metabolic enzyme deficient in multiple different cancers, Proc. Natl. Acad. Sci. USA 93 (1996) 6203–6208.

[17] J.M. Garcia-Castellano, A. Villanueva, J.H. Healey, R. Sowers, C. Cordon-Cardo, A. Huvos, J.R. Bertino, P. Meyers, R. Gorlick, Methylthioadenosine phosphorylase gene deletions are common in osteosarcoma, Clin. Cancer Res. 8 (2002) 782–787.

[18] S.H. Mudd, Enzymatic cleavage of *S*-adenosylmethionine, J. Biol. Chem. 234 (1959) 87–92.

[19] S. Nishimura, Y. Taya, Y. Kuchino, Z. Oashi, Enzymatic synthesis of 3-(3-amino-3-carboxypropyl)uridine in *Escherichia coli* phenylalanine transfer RNA: transfer of the 3-amino-acid-3-carboxypropyl group from *S*-adenosylmethionine, Biochem. Biophys. Res. Commun. 57 (1974) 702–708.

[20] H.G. Williams-Ashman, J. Seidenfeld, P. Galletti, Trends in the biochemical pharmacology of 5′-deoxy-5′-methylthioadenosine, Biochem. Pharmacol. 31 (1982) 277–288.

[21] D. Thomas, A. Becker, Y. Surdin-Kerjan, Reverse methionine biosynthesis from *S*-adenosylmethionine in eukaryotic cells, J. Biol. Chem. 275 (2000) 40718–40724.
[22] A.L. Subhi, P. Diegelman, C.W. Porter, B. Tang, Z.J. Lu, G.D. Markham, W.D. Kruger, Methylthioadenosine phosphorylase regulates ornithine decarboxylase by production of downstream metabolites, J. Biol. Chem. 278 (2003) 49868–49873.
[23] M.K. Chattopadhyay, C.W. Tabor, H. Tabor, Studies on the regulation of ornithine decarboxylase in yeast: effect of deletion in the MEU1 gene, Proc. Natl. Acad. Sci. USA 102 (2005) 16158–16163.
[24] D. Balasundaram, C.W. Tabor, H. Tabor, Spermidine or spermine is essential for the aerobic growth of *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA 88 (1991) 5872–5876.
[25] N. Hamasaki-Katagiri, C.W. Tabor, H. Tabor, Spermidine biosynthesis in *Saccharomyces cerevisiae*: polyamine requirement of a null mutant of the SPE3 gene (spermidine synthase), Gene 187 (1997) 35–43.
[26] L.W. Parks, F. Schlenk, The stability and hydrolysis of *S*-adenosylmethionine; isolation of *S*-ribosylmethionine, J. Biol. Chem. 230 (1958) 295–305.
[27] A.E. Pegg, Inhibition of aminopropyltransferases, Methods Enzymol. 94 (1983) 294–297.
[28] H. Hibasami, R.T. Borchardt, S.Y. Chen, J.K. Coward, A.E. Pegg, Studies of inhibition of rat spermidine synthase and spermine synthase, Biochem. J. 187 (1980) 419–428.
[29] A. Raina, T. Hyvonen, T. Eloranta, M. Voutilainen, K. Samejima, B. Yamanoha, Polyamine synthesis in mammalian tissues. Isolation and characterization of spermidine synthase from bovine brain, Biochem. J. 219 (1984) 991–1000.
[30] I.H. Fox, T.D. Palella, D. Thompson, C. Herring, Adenosine metabolism: modification by *S*-adenosylhomocysteine and 5′-methylthioadenosine, Arch. Biochem. Biophys. 215 (1982) 302–308.
[31] H. Yamanaka, E.O. Kajander, D.A. Carson, Modulation of diphthamide synthesis by 5′-deoxy-5′-methylthioadenosine in murine lymphoma cells, Biochim. Biophys. Acta 888 (1986) 157–162.
[32] M.S. Donoviel, E.T. Young, Isolation and identification of genes activating UAS2-dependent ADH2 expression in *Saccharomyces cerevisiae*, Genetics 143 (1996) 1137–1148.