JOURNAL OF BACTERIOLOGY, Oct. 1994, p. 6407–6409
0021-9193/94/$04.00+0

Vol. 176, No. 20

The Presence of an Active *S*-Adenosylmethionine Decarboxylase Gene Increases the Growth Defect Observed in *Saccharomyces cerevisiae* Mutants Unable To Synthesize Putrescine, Spermidine, and Spermine

DAVID BALASUNDARAM,* QIAO-WEN XIE,† CELIA WHITE TABOR, AND HERBERT TABOR

Laboratory of Biochemical Pharmacology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland 20892

Received 6 June 1994/Accepted 14 August 1994

***Saccharomyces cerevisiae spe1Δ SPE2* mutants (lacking ornithine decarboxylase) and *spe1Δ spe2Δ* mutants (lacking both ornithine decarboxylase and *S*-adenosylmethionine decarboxylase) are equally unable to synthesize putrescine, spermidine, and spermine and require spermidine or spermine for growth in amine-free media. The cessation of growth, however, occurs more rapidly in *spe1Δ SPE2* cells than in *SPE1 spe2Δ* or *spe1Δ spe2Δ* cells. Since *spe1Δ SPE2* cells can synthesize decarboxylated adenosylmethionine (dcAdoMet), these data indicate that dcAdoMet may be toxic to amine-deficient cells.**

Saccharomyces cerevisiae contains the enzymes essential for the biosynthesis of putrescine, spermidine, and spermine (Fig. 1) (4, 6). We previously prepared a null deletion-insertion mutation (*spe2Δ*) in the gene for *S*-adenosylmethionine decarboxylase (AdoMet decarboxylase) which converts *S*-adenosylmethionine (*S*-AdoMet) to decarboxylated adenosylmethionine (dcAdoMet), an intermediate in the biosynthesis of both spermidine and spermine. This mutant requires ca. 15 doublings in amine-free medium before growth stops (2).

In this work, we compare the relative growth of a *SPE1 spe2Δ* strain (which contains a high concentration of putrescine but no spermidine or spermine [2]) with the growth of *spe1Δ spe2Δ* and *spe1Δ SPE2* strains (both of which lack all three amines).

Strains. The strains used are listed in Table 1. The construction of the *spe1Δ::LEU2* deletion-insertion mutant (strain Y359) was carried out by a one-step disruption technique (17). We treated pSPE1U (19), containing the *SPE1* gene derived from the original pSPE-12 supplied by Fonzi and Sypherd (7, 8), with *Bst*EII and *Bgl*II, which deleted a 1,764-bp sequence that included most of the *SPE1* gene. This deletion was replaced with a 2,026-bp *Nar*I-*Hpa*I fragment from YEp352 containing the *LEU2* gene (9). Treatment with *Pvu*II released a 3,162-bp fragment containing the mutated *spe1Δ::LEU2* gene which was used to transform (10) strain 2602, a *SPE1 leu2* strain. LEU^+ recombinants were selected and tested for the absence of ornithine decarboxylase activity. The *spe1Δ spe2Δ* double mutant (Y362) was obtained by sporulation of the diploid Y360 (Table 1) and subsequent tetrad dissection. In this paper, *spe1Δ* = *spe1Δ::LEU2* and *spe2Δ* = *spe2Δ::LEU2* (Table 1). pSPE 2-3 is a plasmid that overproduces the *SPE2* gene product, and its construction has previously been described (3).

Growth experiments. All growth experiments were carried out at 30°C with shaking in air. Strains were grown overnight in YPAD medium (2), collected by centrifugation, washed with water, and resuspended in an equal volume of water. These cells were diluted in H medium (2) containing the necessary supplements to give an initial optical density of 0.01 (see Fig. 2). When the optical density of a culture exceeded 1.0, the culture was diluted 10-fold in the same medium. Data were corrected for these dilutions.

Determination of dcAdoMet. The cells were extracted with trichloroacetic acid, chromatographed on an Aminex A5 column, and eluted with buffer as we previously described for the determination of polyamines (2). UV absorbance was monitored at 254 nm; dcAdoMet was eluted at 50 min. The amount of UV absorbance in the peak was compared with that of synthetic dcAdoMet supplied by Giorgio Stramentinoli. Only free dcAdoMet was measured; i.e., derivatives such as the acetyl dcAdoMet in mammalian cells described by Pegg et al. (16) were not assayed.

Growth rates. Figure 2 shows the growth rates of various strains after transfer from an amine-containing (YPAD) medium to an amine-free purified medium. Both the *SPE1 spe2Δ* strain (that makes putrescine but not spermidine and spermine) and the *spe1Δ spe2Δ* strain (that cannot make putrescine, spermidine, or spermine) required the same number of doublings to reach the depleted state (as measured by the cessation of growth) despite the presence of a high level of putrescine in the *SPE1 spe2Δ* strain.

We also measured the growth rate of the *spe1Δ SPE2* strain. Although the amine profile of this strain is the same as that of the *spe1Δ spe2Δ* strain, i.e., both strains lack all three amines, the effect of amine deficiency occurred significantly earlier in the *spe1Δ SPE2* strain. Thus, the growth of this strain was markedly decreased within the first day after dilution in

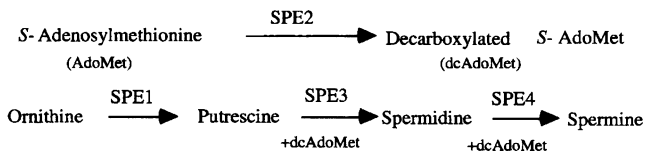

FIG. 1. Diagram of the biosynthesis of putrescine, spermidine, and spermine.

* Corresponding author. Phone: (301) 496-1309. Fax: (301) 402-0240.

† Present address: Department of Medicine, Cornell University Medical School, New York, NY 10021.

TABLE 1. Strains used in this study

Strain	Ploidy	Genotype	Source or reference
2602	Haploid	*MATα ura3-52 his6 leu2*	R. B. Wickner
Y343	Haploid	*MATa trp3 leu2 spe2Δ::LEU2*	2
Y344	Haploid	*MATa ura3-52 his6 leu2 spe2Δ::LEU2*	2
Y359	Haploid	*MATα ura3-52 his6 leu2 spe1Δ::LEU2*	This study
Y360	Diploid	*MATα ura3-52 TRP3 his6 leu2 spe1Δ::LEU2 MATa URA3 trp3 HIS6 leu2 spe2Δ:LEU2*	Y359 × Y343; this study
Y361	Haploid	*MATa ura3-52 his6 leu2 SPE1 SPE2*	Sporulation of Y360
Y362	Haploid	*MATα ura3-52 leu2 spe1Δ::LEU2 spe2Δ::LEU2*	Sporulation of Y360
Y363	Haploid	*MATa ura3-52 his6 leu2 spe1Δ::LEU2*	Sporulation of Y360

purified medium. In contrast, the *spe1Δ spe2Δ* strain continued to grow slowly for 4 to 5 days in amine-free medium before the complete cessation of growth (2). Similar growth curves were obtained when cell counts were measured, indicating that the early cessation of growth in the *spe1Δ SPE2* strain was not the result of the loss of cell viability.

To confirm the deleterious effect of the *SPE2* gene in a polyamine-deficient mutant, we transformed the *spe1Δ spe2Δ* and *spe1Δ SPE2* strains with either a plasmid containing the *SPE2* gene (pSPE 2-3) or a control plasmid (YEp352) and compared the growth curves during amine deprivation. There were very rapid decreases in growth rate (as measured by optical density) for the *spe1Δ SPE2* and *spe1Δ spe2Δ* strains that contained the overproducing pSPE 2-3 compared with the growth rates of the same strains with the vector YEp352 alone (data not shown).

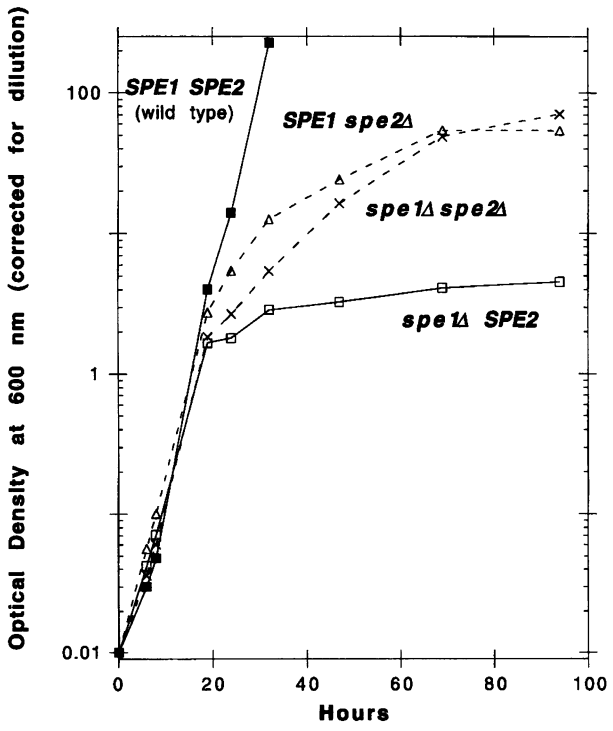

FIG. 2. Growth curves for the *spe1Δ::LEU* mutant Y363 (□), the *spe2Δ::LEU* mutant Y344 (△), the *spe1Δ::LEU spe2Δ::LEU* double mutant Y362 (×), and the *SPE1 SPE2* strain Y361 (■) after transfer from YPAD medium to amine-free H medium. When the optical density of a culture exceeded 1.0, the culture was diluted 10-fold in the same medium. Data were corrected for these dilutions.

TABLE 2. Effects of a multicopy plasmid encoding the *SPE2* gene on the accumulation of dcAdoMet in putrescine-deficient *S. cerevisiae*

Strain	Chromosomal genotype	Plasmid genotype	dcAdoMet (nmol/g [wet wt])[a]
Y362/YEp352	*spe1Δ spe2Δ*	Vector only	Not detected
Y362/pSPE 2-3	*spe1Δ spe2Δ*	*SPE2*	170
Y363/YEp352	*spe1Δ SPE2*	Vector only	110
Y363/pSPE 2-3	*spe1Δ SPE2*	*SPE2*	860
2602	*SPE1 SPE2*	No plasmid	Not detected

[a] After growth in polyamine-deficient medium for 2 to 3 days. The limit of detection was 10 nmol per g (wet wt).

dcAdoMet accumulates in *spe1Δ* strains that contain a wild-type *SPE2* gene. After incubation for 2 to 3 days in amine-deficient media, marked increases in the amounts of dcAdoMet in *spe1Δ SPE2* and *spe1Δ SPE2*/pSPE 2-3 cells were found (Table 2). This finding is consistent with previous work on the accumulation of dcAdoMet in *S. cerevisiae* deficient in spermidine synthase (5) and in other cells and urine (1, 11–16, 18) after the inhibition of ornithine decarboxylase by treatment with difluoromethylornithine. The amount of dcAdoMet in *SPE1 SPE2* cells was too low to be detected by our assays (<10 nmol/g [wet weight]). While our results do not explain the mechanism of toxicity observed in our experiments, one possibility is that histone acetylation is inhibited by dcAdoMet, as previously shown by Pegg et al. (16) in vitro with nuclear extracts from rat livers.

In most studies on the physiologic role of polyamines, the levels of polyamines have been changed either by the use of inhibitors or by mutations. In view of the results reported in this paper, the clinical effects of inhibitors might be influenced by the amounts of active AdoMet decarboxylase or its enzymatic products in amine-deficient cells. Consistent with this possibility is a recent report (1) that the increased susceptibility of some strains of trypanosomes from rats treated with α-difluoromethylornithine could be correlated with increased combined pools of *S*-AdoMet and AdoMet decarboxylase in the parasites. Thus, our results may be relevant to the therapeutic efficiency of the inhibitors of ornithine decarboxylase and dcAdoMet that are being used as potential antitumor or trypanocidal agents.

We acknowledge the assistance of Ain Robinson, a participant in the Howard Hughes Medical Institute-NIH/Montgomery County Public Schools Internship program (1990), in the preparation of various mutants and plasmids.

REFERENCES

1. **Bacchi, C. J., J. Garofalo, M. Ciminelli, D. Rattendi, B. Goldberg, P. P. McCann, and N. Yarlett.** 1993. Resistance to DL-α-difluoromethylornithine by clinical isolates of *Trypanosoma brucei rhodesiense*. Role of *S*-adenosylmethionine. Biochem. Pharmacol. **46**:471–481.
2. **Balasundaram, D., C. W. Tabor, and H. Tabor.** 1991. Spermidine or spermine is essential for the aerobic growth of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **88**:5872–5876.

3. **Balasundaram, D., C. W. Tabor, and H. Tabor.** 1993. Oxygen toxicity in a polyamine-depleted *spe2*Δ mutant of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **90**:4693–4697.
4. **Cohn, M. S., C. W. Tabor, and H. Tabor.** 1978. Isolation and characterization of *Saccharomyces cerevisiae* mutants deficient in *S*-adenosylmethionine decarboxylase, spermidine, and spermine. J. Bacteriol. **134**:208–213.
5. **Cohn, M. S., C. W. Tabor, and H. Tabor.** 1979. Mutants of *Saccharomyces cerevisiae* defective in the biosynthesis of polyamines from *S*-adenosylmethionine, p. 91–93. *In* E. Usdin, R. T. Borchardt, and C. R. Creveling (ed.), Transmethylation. Elsevier/North-Holland, New York.
6. **Cohn, M. S., C. W. Tabor, and H. Tabor.** 1980. Regulatory mutations affecting ornithine decarboxylase activity in *Saccharomyces cerevisiae*. J. Bacteriol. **142**:791–799.
7. **Fonzi, W. A.** 1989. Biochemical and genetic characterization of the structure of yeast ornithine decarboxylase. Biochem. Biophys. Res. Commun. **162**:1409–1416.
8. **Fonzi, W. A., and P. S. Sypherd.** 1985. Expression of the gene for ornithine decarboxylase of *Saccharomyces cerevisiae* in *Escherichia coli*. Mol. Cell. Biol. **5**:161–166.
9. **Hill, J. E., A. M. Myers, T. J. Koerner, and A. Tzagoloff.** 1986. Yeast/E. coli shuttle vectors with multiple unique restriction sites. Yeast **2**:163–167.
10. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
11. **Janne, J., and L. Alhonen-Hongisto.** 1989. Inhibitors of ornithine decarboxylase: biochemistry and applications, p. 59–85. *In* S.-I. Hayashi (ed.), Ornithine decarboxylase: biology, enzymology, and molecular genetics. Pergamon Press, Elmsford, N.Y.
12. **Mamont, P. S., C. Danzin, J. Wagner, M. Siat, A. M. Joder-Ohlenbusch, and N. Claverie.** 1982. Accumulation of decarboxylated *S*-adenosy-L-methionine in mammalian cells as a consequence of the inhibition of putrescine biosynthesis. Eur. J. Biochem. **123**:499–504.
13. **Marton, L. J., and P. L. Lee.** 1975. More sensitive automated detection of polyamines in physiological fluids and tissue extracts with *o*-phthalaldehyde. Clin. Chem. **21**:1721–1724.
14. **Pegg, A. E.** 1984. The role of polyamine depletion and accumulation of decarboxylated *S*-adenosylmethionine in the inhibition of growth of SV-3T3 cells treated with α-difluoromethylornithine. Biochem. J. **224**:29–38.
15. **Pegg, A. E., H. Poso, K. Shuttleworth, and R. A. Bennett.** 1982. Effect of inhibition of polyamine synthesis on the content of dAdoMet. Biochem. J. **202**:519–526.
16. **Pegg, A. E., R. S. Wechter, R. S. Clark, L. Wiest, and B. G. Erwin.** 1986. Acetylation of decarboxylated *S*-adenosylmethionine by mammalian cells. Biochemistry **25**:379–384.
17. **Rothstein, R. J.** 1983. One-step gene disruption. Methods Enzymol. **101**:202–211.
18. **Wagner, J., C. Danzin, and P. Mamont.** 1982. Reversed-phase ion-pair liquid chromatographic procedure for the simultaneous analysis of *S*-adenosylmethionine, its metabolites and the natural polyamines. J. Chromatogr. **227**:349–368.
19. **Xie, Q.-W., C. W. Tabor, and H. Tabor.** 1990. Ornithine decarboxylase in *Saccharomyces cerevisiae*: chromosomal assignment and genetic mapping of the *SPE1* gene. Yeast **6**:455–460.