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Methionine Adenosyltransferase and Ethionine Resistance in *Saccharomyces cerevisiae*

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The methionine adenosyltransferase is repressed in *Saccharomyces cerevisiae* during growth in the presence of excess methionine. The relationship of this repression to the level of intracellular *S*-adenosylmethionine is discussed. In conjunction with these studies, an ethionine-resistant mutant has been investigated which has a low level of methionine adenosyltransferase under all conditions tested. The mechanism of ethionine resistance in the latter strain apparently depends on its inability to form large quantities of intracellular *S*-adenosylethionine. With respect to the methionine adenosyltransferase, there is no apparent interaction between ethionine-resistant and ethionine-sensitive alleles when both are present in the heterozygous diploid.

The reaction synthesizing *S*-adenosylmethionine from L-methionine and adenosine triphosphate (ATP) is catalyzed by methionine adenosyltransferase (ATP:L-methionine-*S*-adenosyltransferase EC 2.5.1.6). The enzyme in yeast will also catalyze the formation of *S*-adenosylethionine from the amino acid analogue, ethionine (23). The mechanism of the methionine adenosyltransferase and its requirements have been extensively investigated (1, 2, 16, 17). The enzyme has been monitored in a variety of microorganisms (8, 17, 21), as well as in liver extracts (6, 18). Under certain conditions, methionine adenosyltransferase from yeast has been shown to be induced in the presence of low concentrations of methionine (20).

Ethionine acts as a competitive analogue with methionine for protein synthesis (15) and represses the formation of sulfur-containing amino acids (22, 28). In some cases, the cell is capable of transferring ethyl groups from *S*-adenosylethionine onto compounds which are normally methylated (19, 28). Such ethylation may result in either inhibition or death of the cell.

Several mutations have been described which affect the resistance of a cell to ethionine. A mutation in *Neurospora* affects the ability of the cells to distinguish between methionine and ethionine at the level of protein synthesis (14). Recessive mutations have been described which have been shown to be general permease mutations blocking the uptake of ethionine and other amino acid analogues (25, 29). Others have been described in which ethionine resistance is due to an alteration in methionine biosynthesis or in the regulation of the methionine pathway (3-5).

A mutation for ethionine resistance in *Saccharomyces cerevisiae* has been described which is specific for resistance to ethionine (27). Initial studies showed that it was not a permease mutation. There was little transethylation in the resistant strain. The results suggested that the mutation altered the fate of ethionine either by degradation of the amino acid analogue or by converting *S*-adenosylethionine to compounds which were not maintained internally. In addition, the absence of *S*-adenosylethionine accumulation in the mutant suggested that resistance might be due to reduced activity of the methionine adenosyltransferase. The latter would screen the ethionine from conversion to *S*-adenosylethionine and protect the cell from subsequent transethylations.

Recent studies performed to establish the mechanism of ethionine resistance in this strain strongly suggest that the mutation is a regulatory alteration in *S*-adenosylmethionine synthesis. These studies, together with the regulatory aspects of *S*-adenosylmethionine synthesis, are the subject of this report.

MATERIALS AND METHODS

Chemicals, media, and organisms. *S*-adenosyl-L-ethionine was prepared in this laboratory ac-

cording to the technique of Schlenk et al. (23). All other chemicals were obtained from commercial sources. Methionine and ethionine in the L-form were employed throughout. Yeast complete medium (YCM) consisted of 2% glucose, 2% tryptone, and 1% yeast extract. The composition of medium B, a synthetic glucose-salts medium, has been previously described (26). In all experiments where uracil mutants were employed, the pyrimidine was added to medium B at a concentration of 0.2 mM.

The derivation of strains 3701B (*ura*) and R3720 (*ura*, *et*^{r2}) of *S. cerevisiae* has been previously described (27). Strain RJB21 (*ade-2*, *et*^{r2}) is a haploid segregant of a cross between strains R3720 and JB4 (*met*, *ade-2*). Strains JB4 and ET48 (*met-3*) were obtained from R. K. Mortimer, Univ. of California, Berkeley.

Strains to be mated were inoculated together into 5 ml of YCM broth and incubated overnight at 30 C. Cells were spread on medium B agar without supplements to select for diploids. Prior to sporulation, diploid strains were grown in YCM broth overnight at 30 C. The cells were washed and spread on the surface of Fowell's medium (7) and incubated at least 5 days at 30 C or until ascospores were evident upon observation by microscope. When sporulation was complete, the cells were suspended in sterile water at 10^6 cells per ml and snail digestive juice (13) was added to a final dilution of 1:200. The cells and enzyme were incubated at room temperature with shaking for 20 min or until asci walls were broken. The solution was diluted to stop enzyme action, and the ascospores were spread on the surface of YCM agar plates. The haploid colonies were removed from these plates for replica plating. Among the haploids verified by mating tests, a 2:2 ratio of ethionine resistance to ethionine sensitivity was obtained as previously described (27). Suitable haploids were mated to obtain the following three diploids for further study: 3701B/ET48 (+/+), homozygous for the sensitive marker, R3720/ET48 (*et*^{r2}/+), heterozygous for the resistant marker, and R3720/RJB21 (*et*^{r2}/*et*^{r2}), homozygous with respect to ethionine resistance.

Growth on supplements. Growth responses of diploid and haploid strains were determined in medium B broth and in medium B containing various amounts of ethionine and *S*-adenosylethionine. Growth was carried out on a shaker at 30 C and was recorded on a Klett-Summerson photoelectric colorimeter using the blue filter (400 to 465 nm). Before exposure to growth supplements, the cells were grown in YCM for 24 hr, then preadapted to medium B for two generations. After adaptation, the cell concentration was adjusted to 15 Klett units (approximately 0.05 mg dry weight of cells per ml) in fresh medium B and ethionine or *S*-adenosylethionine was added.

Enzyme purification. Cells used for the assay of methionine adenosyltransferase were grown in either YCM broth or medium B with various supplements. The cells were collected, washed twice in cold distilled water, then resuspended in two volumes of 0.1 M potassium phosphate buffer at *p*H 6.7 containing 1 mM methionine, and were broken in a French pressure cell at 20,000 psi. Cell debris was removed by centrifugation at 4 C (27,000 × *g*, 30 min). The crude extract was then partially purified by modifications of the procedures of Mudd and Cantoni (17) and Greene (9). All operations were carried out in the cold. The extract was diluted 1:2.5 with the above buffer. Bentonite at 30 mg per ml was added slowly with stirring. The solution was stirred for 20 min, and then the bentonite was removed by centrifugation (30,000 × *g*, 30 min). An ammonium sulfate fractionation was performed, and the fraction which precipitated between 60 and 90% of saturation was collected and saved in a frozen state until used. The paste was resuspended in 0.02 M tris(hydroxymethyl)-aminomethane (Tris) buffer at *p*H 7.6 and dialyzed overnight against 200 volumes of the same buffer immediately before use. Protein concentration was determined by the method of Groves et al. (11).

Methionine adenosyltransferase assay. The reaction mixture for the synthesis of *S*-adenosylmethionine and *S*-adenosylethionine contained per milliliter: 200 μmoles of Tris buffer at *p*H 8.5, 10 μmoles of ATP, 5 μmoles of reduced glutathione, 50 μmoles of potassium chloride, 15 μmoles of magnesium chloride, 10 μmoles of methionine containing 0.5 μCi of methionine-$^{14}CH_3$, and 5 mg of protein. When the enzyme was tested for its ability to synthesize *S*-adenosylethionine, 10 μmoles of ethionine containing 0.5 μCi of ethionine-$^{14}CH_2CH_3$ was substituted for methionine in the reaction mixture. The reaction mixture was incubated at 37 C. The short column technique of Shapiro and Ehninger (24) was modified for the assay of *S*-adenosylmethionine and *S*-adenosylethionine. Columns (0.5 by 3.0 cm) of Dowex-50W-4X 50 to 100 ion-exchange resin in the Li^+ form were employed throughout. After charging of the columns, 0.2 ml of the reaction mixture was applied. Unreacted methionine or ethionine was removed with 10 ml of distilled water, and the *S*-adenosylmethionine or *S*-adenosylethionine was eluted with 10 ml of 3 N NH_4OH. The radioactivity was measured in toluene–ethanol (1:1) plus 0.4% 2,5-diphenyloxazole with a Nuclear-Chicago Unilux III liquid scintillation spectrometer.

Methionine adenosyltransferase activity after growth in different media. Cells used for the study of methionine adenosyltransferase under different growth conditions were first cultured in YCM broth for 24 hr at 30 C. The cells were harvested and washed, and resuspended at 30 Klett units in YCM, medium B, or medium B supplemented with 1 mM and 5 mM methionine. Growth was continued for three generations at 30 C. The cells were then harvested and the extracts were prepared as earlier described. A small amount of the crude cellular extract was dialyzed and assayed with the treated preparations. The proportionality between the enzyme activities of cells grown under the various conditions remained unaltered by the purification procedure.

Accumulation of S-adenosylmethionine and S-adenosylethionine in haploid cells. Cells used for the study of internal concentrations of *S*-adenosylmethionine and *S*-adenosylethionine were grown

overnight in YCM. The cells were washed and resuspended at 20 Klett units in 500 ml of medium B. After one generation of growth at 30 C with shaking, concentrations of 0.1 mM, 1.0 mM, and 5.0 mM methionine or ethionine were added to the cultures and growth was continued until 80 Klett units were reached. In the presence of ethionine, the sensitive strain took about twice as long as the resistant strain to double and then ceased growing. The cells were extracted with perchloric acid and *S*-adenosylmethionine or *S*-adenosylethionine assayed by the technique of Shapiro and Ehninger (24).

RESULTS

Growth response of haploid strains to ethionine and S-adenosylethionine. The net increase in weight of R3720 and 3701B in liquid medium B supplemented with various concentrations of ethionine is compared to noninhibited growth in Fig. 1. During a 10-hr interval, the sensitive strain is inhibited 90%, and the resistant strain is reduced approximately 25% in growth in 0.04 mM ethionine.

Previous work (27) suggested that the resistance of R3720 was due in part to either a reduction in the cell's ability to produce *S*-adenosylethionine, or its increased ability to hydrolyze *S*-adenosylethionine to nontoxic products. If the resistant strain produces less *S*-adenosylethionine, a lower level of *S*-adenosylmethionine production might be expected as well. If the latter were true, one would expect R3720 to be more sensitive than 3701B to inhibition by *S*-adenosylethionine. Data from growth of both the resistant and sensitive haploids in the presence of various concentrations of *S*-adenosylethionine are shown in Fig. 2. At all concentrations, R3720 proved to be slightly more sensitive than the ethionine-sensitive strain.

Intracellular accumulation of S-adenosylethionine and S-adenosylmethionine. The amount of *S*-adenosylethionine which accumulates in the resistant strain after one generation growth in various concentrations of ethionine is compared to the sensitive strain (Fig. 3; shaded symbols). It is evident that R3720 accumulates only a very small amount of *S*-adenosylethionine as compared to 3701B. Based on earlier reports (22, 27) it is probably safe to assume that the wild-type levels of *S*-adenosylethionine measured after growth in 1 mM and 5 mM ethionine contained only negligible amounts of endogenous *S*-adenosylmethionine. The levels of *S*-adenosylmethionine accumulated under identical conditions, with methionine replacing ethionine, are also shown (Fig. 3; open symbols). The ethionine-sensitive strain accumulates approximately 3.5 times more *S*-adenosylmethionine than the et^{r2} strain. Although this is not as great a difference as that observed with *S*-adenosyl-

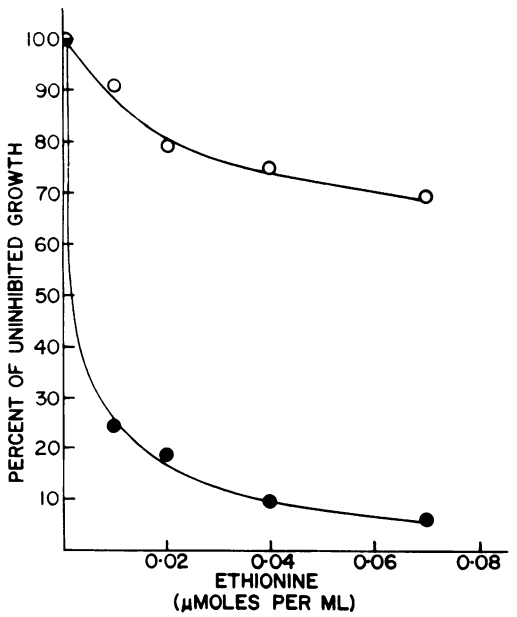

FIG. 1. *Percentage of uninhibited growth by haploid strains 3701B (+) (●) and R3720 (et^{r2}) (○) after cultivation in liquid medium B supplemented with various concentrations of ethionine. The growth period was 10 hr.*

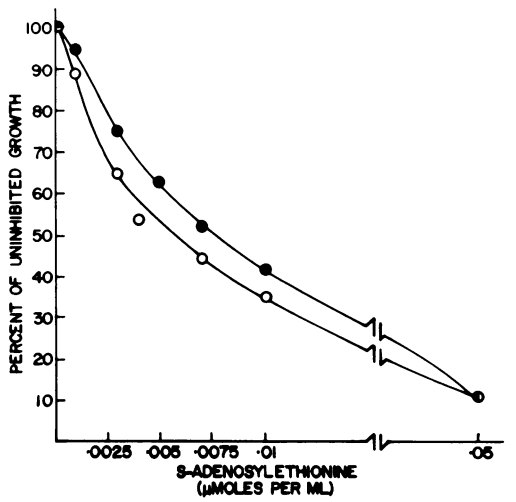

FIG. 2. *Percentage of uninhibited growth by haploid strains 3701B (+) (●) and R3720 (et^{r2}) (○) after cultivation in liquid medium B supplemented with various concentrations of S-adenosylethionine. The growth period was 10 hr.*

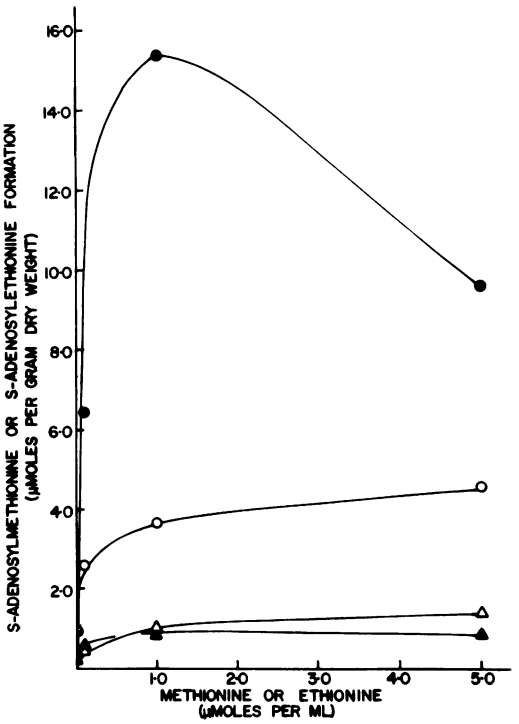

FIG. 3. *Concentration of S-adenosylmethionine and S-adenosylethionine in haploid cells grown in medium B supplemented with various concentrations of methionine and ethionine.* (○) *Methionine-supplemented 3701B* (+); (●) *ethionine-supplemented 3701B* (+); (△) *methionine-supplemented R3720* (et^{r2}); (▲) *ethionine-supplemented R3720* (et^{r2}).

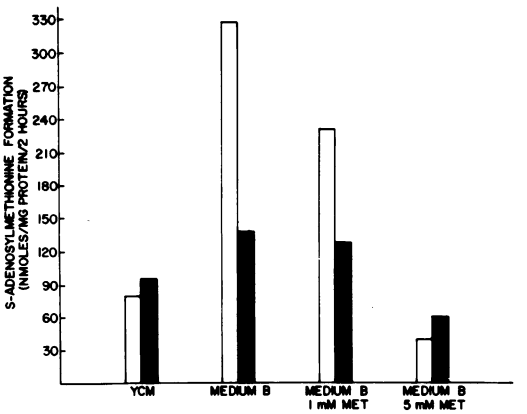

FIG. 4. *Formation of S-adenosylmethionine by extracts from 3701B* (+) *(open bar) and R3720* (et^{r2}) *(shaded bar) grown in yeast complete medium, in medium B, and in medium B supplemented with 1 mM and 5 mM methionine (MET).*

ethionine accumulation, it nevertheless shows that both the *S*-adenosylmethionine and *S*-adenosylethionine balance are affected by this mutation.

Levels of methionine adenosyltransferase activity in R3720 and 3701B. Since both *S*-adenosylethionine and *S*-adenosylmethionine were accumulated in lesser quantities in the et^{r2} strain, the possibility existed that the compound was not made efficiently in the resistant strain due to a low activity of the methionine adenosyltransferase. A comparison of the activities of the methionine adenosyltransferase in the resistant and sensitive haploids is shown for a variety of growth conditions in Fig. 4. Under these experimental conditions, it was found that the level of enzyme in the sensitive strain was highest after growth in a minimal salts medium. The presence of either 1 mM or 5 mM methionine in this medium led to a reduction in the level of enzyme. The activity in YCM was also repressed below that found in the unsupplemented minimal salts medium. In contrast, the ethionine-resistant strain was only moderately affected by the different media or by the presence of methionine in the medium. Under optimal conditions for production of enzyme (i.e., growth in unsupplemented medium B) the level of methionine adenosyltransferase in the sensitive strain greatly exceeds that level found in R3720.

Growth response of diploid strains to ethionine. The net increase in weight after 10 hr of growth in various concentrations of ethionine is compared to uninhibited cells in three diploid strains in Fig. 5. The strain homozygous for for ethionine sensitivity (+/+) is completely inhibited by concentrations of ethionine above 0.04 mM. However, the strain homozygous for ethionine-resistance (et^{r2}/et^{r2}) shows only limited inhibition of growth by the analogue. The response of these two diploids roughly corresponds to the response of R3720 and 3701B under comparable conditions (Fig. 1). However, the heterozygous diploid ($et^{r2}/+$) shows an intermediate level of resistance to ethionine when compared to the other two diploids. This kind of partial dominance probably results from a gene dosage effect.

Levels of methionine adenosyltransferase activity in three diploids. To test whether the apparent partial dominance (Fig. 5) would be reflected in the methionine adenosyltransferase levels of the heterozygous diploid, the enzyme levels of three diploids were compared after growth in minimal salts medium. The specific activities of this enzyme in the three diploid strains are recorded in Table 1.

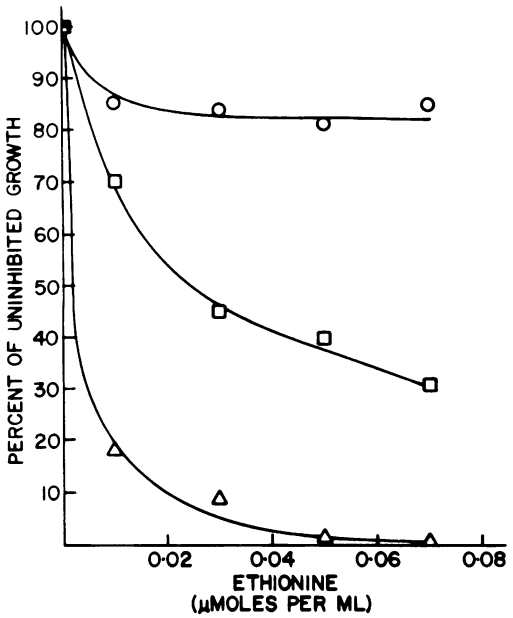

FIG. 5. *Percentage of uninhibited growth by diploid strains R3720/RJB21 (et^{r2}/et^{r2}) (○), R3720/ET48 ($et^{r2}/+$) (□), and 3701B/ET48 (+/+) (△) after cultivation in liquid medium B supplemented with various concentrations of ethionine. The growth period was 10 hr.*

TABLE 1. *Methionine adenosyltransferase activities of ethionine-resistant and ethionine-sensitive haploids and diploids after growth in medium B*[a]

Strain	Genotype	*S*-adenosylmethionine formed: Specific activity[b]	*S*-adenosylethionine formed: Specific activity[b]
R3720/RJB21	et^{r2}/et^{r2}	1.7	0.53
R3720/ET48	$et^{r2}/+$	3.5	0.88
3701B/ET48	+/+	4.7	1.47
R3720	et^{r2}	1.6	
3701B	±	4.9	

[a] Cells cultured and enzyme prepared as described in Materials and Methods.

[b] Initial velocity of reaction as nanomoles of *S*-adenosylmethionine or *S*-adenosylethionine formed per milligram of protein per minute.

In fact, the strain heterozygous for the ethionine-resistant marker shows an intermediate level of enzyme activity, approximately the average of the activities of the two homozygous strains. The activities of the haploid strains under these conditions are included in the table for comparative purposes. The homozygous diploids, (et^{r2}/et^{r2}, and +/+) possess essentially the same activity as the haploid strains (et^{r2} and +). The response of the methionine adenosyltransferase to ethionine as a substrate was also compared in the three diploids. The ability of the strains to convert ethionine to *S*-adenosylethionine is 25 to 30% of their ability to synthesize *S*-adenosylmethionine from methionine. The relationship remains the same in both mutants and sensitive strains.

DISCUSSION

Earlier studies have shown that ethionine is not retained in the et^{r2} strain, but is rapidly converted to compounds which are excreted from the cell (27). It was not possible to distinguish between two probable mechanisms at that time. It appeared that either ethionine or *S*-adenosylethionine, or both, were converted to nontoxic or excretable products, or both. It is now clear that the conversion to *S*-adenosylethionine is not requisite for resistance to ethionine. The synthesis of *S*-adenosylethionine is not only low in the mutant strain, but the level of the methionine adenosyltransferase in the et^{r2} mutant is also correspondingly low. These observations are consistent with those showing a decreased intracellular *S*-adenosylmethionine pool and an increased *S*-adenosylethionine sensitivity in the et^{r2} strain. The slightly larger quantity of ethylthioadenosine formed by the ethionine-resistant strain (27) was apparently not significant.

The yeast system described here appears to share much in common with that recently described in *Escherichia coli* (10, 12). The methionine adenosyltransferase of *E. coli* is also repressed during growth in the presence of methionine. Two ethionine-resistant mutants (10) with low methionine adenosyltransferase levels have also been described. Other parallels will perhaps be discovered.

S-adenosylmethionine synthesis in strain 3701B appears to be a repressible system. The fact that *S*-adenosylmethionine is the product of the methionine adenosyltransferase reaction, and the fact that one observes an inverse relationship between the level of the enzyme and its product, tempts one to assume that *S*-adenosylmethionine is the corepressor molecule of methionine adenosyltransferase synthesis. And as has been previously suggested (3), it is not improbable that the corepressor of the sequence catalyzing the synthesis of methionine is also *S*-adenosylmethionine. It is apparent that if a repressible system is indeed operating in *S*-adenosylmethionine biosyn-

thesis, the response of the et^{r2} mutant to normal regulatory controls has been greatly altered. The absence of depression is not alleviated by introduction of the sensitive allele (heterozygous diploid). Since the activity of the latter diploid appeared to be an average of the homozygous resistant and sensitive activities, with no obvious regulatory interaction between alleles, it is probable that the effect of et^{r2} is at or near the site of the methionine adenosyltransferase gene.

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