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Regulatory Mutations Affecting Ornithine Decarboxylase Activity in *Saccharomyces cerevisiae*

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We isolated several strains of *Saccharomyces cerevisiae* containing mutations mapping at a single chromosomal gene (*spe10*); these strains are defective in the decarboxylation of L-ornithine to form putrescine and consequently do not synthesize spermidine and spermine. The growth of one of these mutants was completely eliminated in a polyamine-deficient medium; the growth rate was restored to normal if putrescine, spermidine, or spermine was added. *spe10* is not linked to *spe2* (adenosylmethionine decarboxylase) or *spe3* (putrescine aminopropyltransferase [spermidine synthase]). *spe10* is probably a regulatory gene rather than the structural gene for ornithine decarboxylase, since we isolated two different mutations which bypassed *spe10* mutants; these were *spe4*, an unlinked recessive mutation, and *spe40*, a dominant mutation linked to *spe10*. Both *spe4* and *spe40* mutants exhibited a deficiency of spermidine aminopropyltransferase (spermine synthase), but not of putrescine aminopropyltransferase. This suggests that ornithine decarboxylase activity is negatively controlled by the presence of spermidine aminopropyltransferase.

The three polyamines putrescine, spermidine, and spermine are widely distributed in nature (8), and all three are present in *Saccharomyces cerevisiae* (2, 10, 13). Recent studies with *spe2* mutants of yeast (lacking adenosylmethionine decarboxylase, spermidine, and spermine, but still containing putrescine) (2) have shown that spermidine and spermine are not essential for survival, although they are required for optimal growth. Specific requirements for spermidine or spermine were demonstrated for meiotic sporulation and for the maintenance of the killer plasmid (2, 3).

In order to block synthesis of all three polyamines, we searched for mutants lacking ornithine decarboxylase, a particularly interesting enzyme because of the rapid changes in its levels resulting from growth stimulation in eucaryotic systems (8). Yeast mutants isolated previously (13) which had decreased levels of ornithine decarboxylase were able to grow slowly and synthesize trace levels of polyamines. We have now isolated a mutant which contains no detectable ornithine decarboxylase and is completely deficient in putrescine, spermidine, and spermine; this mutant has an absolute polyamine requirement for growth.

MATERIALS AND METHODS

Media. YPAD (a rich medium containing sufficient amounts of polyamines to allow normal growth of mutants deficient in polyamine biosynthesis), SD (a minimal medium, pH 5, containing no polyamines), sporulation medium, MB medium (for assay of the killer phenotype), and H medium (complete minimal medium, pH 7, containing most amino acids, uracil, adenine, but no polyamines) were prepared as described previously (3). All solid media used to test for growth effects of polyamines were prepared with Difco Bacto-Agar, which contains no polyamines. For sterilization, all liquid media were filter sterilized, and agar was boiled, since the steam used in most autoclaves is contaminated with amine-containing additives which stimulated growth of *spe10* mutants. This was especially true for media at acid pH values (pH of 5 or lower), which can readily pick up amines from steam during autoclaving.

Preparation of amine-deficient strains. In testing organisms for the effect of polyamines on growth, strains were depleted of amines by subjecting them to two successive single-colony isolations on polyamine-deficient medium. The organisms were then streaked onto the same medium with or without 10^{-4} M spermidine. Differences in growth could be noted after 2 days at 26°C.

Mutagenesis and screening method. Mutagenesis was performed with 0.02% *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (5). Mass screening of crude extracts of yeast (grown at 26°C on YPAD plates) for ornithine decarboxylase was usually carried out by a qualitative assay in 96-well Falcon plates (method A). Each well contained 100 μl of the assay buffer (2 mM dithiothreitol, 10 mM potassium phosphate, pH 7.5, 1 mM $MgCl_2$, 0.1 mM pyridoxal 5-phosphate, 0.1 mM EDTA [buffer A]), to which 0.5 mg of zymolyase 5000 (Kirin Brewery Co., Takafaki, Japan) per ml was added. Cells from a single clone were suspended in each well,

with approximately equal densities in all wells. After incubation at 37°C for 1 h, 1.1 nmol (in 25 μl) of L-[*carboxy*-^{14}C]ornithine (56.3 μCi/μmol; New England Nuclear Corp.) was added to each well, $Ba(OH)_2$-impregnated filter paper was placed over the plate to trap $^{14}CO_2$, and incubation was carried out for 4 h at 37°C. Filter papers were screened for potential mutants by autoradiography (2, 12). A modified method (method B) was occasionally used, in which the cells were intact (treatment with zymolyase was omitted), but this method was less convenient for identifying mutants.

Genetic analysis. Complementation studies, tetrad analyses, and other genetic techniques were performed by standard methods (6). Strains are listed in Table 1.

Enzyme assays. For the quantitative assay of ornithine decarboxylase in vitro, cells growing exponentially under the conditions specified for a given experiment were harvested and suspended in buffer A (2 ml of buffer A per g [wet weight] of yeast) at 4°C. The suspension was passed through a French pressure cell (Aminco) at 20,000 lb/in^2, cooled to 4°C, and centrifuged at 15,000 × *g* for 15 min. The supernatant fluid was dialyzed against buffer A overnight at 4°C (1 ml [total volume] of extract and 500 ml of buffer). The assay mixture (in a scintillation vial) contained cell extract, buffer A, and bovine serum albumin (200 μg/ml) in a final volume of 0.4 ml. The reaction was started by adding 1 μCi (in 10 μl) of L-[*carboxy*-^{14}C]ornithine (56.3 mCi/mmol). The vial was closed tightly with a cap which contained a piece (2 by 2 cm) of Whatman no. 3 filter paper impregnated with 20 μl of 1 M Hyamine hydroxide. The vial was incubated for 30 min at 37°C, and the reaction was stopped by adding 0.2 ml of 1 M KH_2PO_4. After the vial was shaken for 1 h, trapped $^{14}CO_2$ on the filter paper was determined after the paper was transferred to another vial containing 5 ml of scintillation fluid; 1 U was defined as the amount of enzyme that formed 1.0 pmol of $^{14}CO_2$ per min. This assay was based on previously published procedures (7). $^{14}CO_2$ production was linear

TABLE 1. *Strain list*

Strain	Genotype	Relevant phenotype	Source or reference
AN33	α *thr1 arg1*		R. B. Wickner
J25	α *thr1 arg1*	Increased ornithine decarboxylase	Mutagenesis of AN33
68-C	α *thr1 arg1*	Increased ornithine decarboxylase	Mutagenesis of J25
M57	α *thr1 arg1 spe10-1*	Poor growth on YPAD	Mutagenesis of AN33
K14	α *thr1 arg1 spe10-2*	Poor growth on YPAD	Mutagenesis of J25
M4	α *thr1 arg1 spe10-3*	Poor growth on YPAD	Mutagenesis of J25
6A-30	α *thr1 arg1 spe10-4*		Mutagenesis of 68-C
72-8D	a *ade2 leu2 ura3 spe2-4*		Reference 2
200	a *lys10*		R. B. Wickner
81	a *thr1 arg1 lys2 kex2*		R. B. Wickner
53	a *ade1*		R. B. Wickner
73-7C	α *ade1 spe10-1*		M57 × 53
75-16A	a *lys2 spe10-1*		73-7C × 81
75-19A	a *thr1 spe10-1*		73-7C × 81
81-1C	α *thr1 ade1 spe10-2*		K14 × 53
81-5C	a *ade1 spe10-2*		K14 × 53
81-12A	a *arg1 spe10-2*		K14 × 53
82-1B	α *thr1 arg1 spe10-3*		M4 × 53
85-5B	a *ade1 spe10-3*		82-1B × 53
85-6A	a *thr1 spe10-3*		82-1B × 53
85-7C	α *thr1 arg1 spe10-3*		82-1B × 53
87-4A	a *ade2 arg1 spe10-3*		82-1B × 72-8D
105-7	α *lys10 spe10-3*		85-7C × 200
73-7CB	α *ade1 spe10-1 spe40-1*		Selected from 73-7C
81-1CB	α *thr1 ade1 spe10-2 spe40-2*		Selected from 81-1C
82-1BB	α *thr1 arg1 spe10-3 spe4-1*		Selected from 82-1B
85-5B × 85-7C	Diploid, homozygous for *spe10-3*	No auxotrophic requirements	
86-10C	a *thr1 spe4-1*		82-1BB × 53
86-9C	α *arg1 spe4-1*		82-1BB × 53
127-2B	α *arg1 spe10-1 spe4-1*		86-9C × 75-19A
127-4D	a *arg1 spe10-1 spe4-1*		86-9C × 75-19A
5 × 47	Diploid K^- R^-	Testor strain for killer phenotype; sensitive to the killer toxin	5 (a *his5-35 trp5*) × 47 (α *ura3*)
A364A	a *ade1 ade2 his7 lys2 aro1 ura1 gal1* K^+ R^+	Able to kill sensitive strains (e.g., 5 × 47)	R. B. Wickner

with respect to time and enzyme concentration.

Assays of *S*-adenosylmethionine decarboxylase and protein were performed as previously described (2). The isotopic assays of putrescine aminopropyltransferase (spermidine synthase) (1) and spermidine aminopropyltransferase (spermine synthase) were performed as follows. Crude extracts (1 ml) of strains to be assayed were dialyzed overnight against 500 ml of 50 mM potassium phosphate (pH 8.2) containing 1 mM EDTA (buffer B). The assay mixture (total volume, 160 μl) contained enzyme (crude extract) and 100 nmol of decarboxylated *S*-adenosylmethionine (1) in buffer B. The reaction was started by adding 0.42 μCi (in 40 μl) of [1,4-^{14}C]putrescine dihydrochloride (4.2 μCi/μmol; New England Nuclear Corp.) or 0.47 μCi (in 40 μl) of [*tetramethylene*-1,4-^{14}C]spermidine trihydrochloride (4.7 μCi/μmol; New England Nuclear Corp.). After incubation at 37°C for 2 h, 2 ml of 10% trichloroacetic acid was added. After centrifugation at 4°C, the supernatant fluid was extracted twice with 6 ml of diethyl ether. Residual ether was removed under a vacuum. Samples were placed on a chromatographic column (Durrum DC-6A) which was used for automated amine analysis (12), and the eluate was collected in 5-ml fractions. The formation of [^{14}C]spermidine from [^{14}C]putrescine or [^{14}C]spermine from [^{14}C]spermidine was detected by the elution of ^{14}C at the position of spermidine of spermine; 1 U was defined as the amount of enzyme which formed 1 pmol of [^{14}C]spermidine or [^{14}C]spermine per min.

Analysis of strains for polyamines. Depleted strains prepared by growth on agar medium (H or SD medium) were grown at 22°C in the same polyamine-deficient liquid medium to an absorbance at 650 nm of 0.5 to 1. Cells were then analyzed for putrescine, spermidine, and spermine by the automated rapid elution system previously described (4, 9). In this system, spermidine (eluting at 60 min) was well separated from decarboxylated *S*-adenosylmethionine (eluting at 90 min); spermine eluted at 132 min. In some instances, putrescine was also determined by a modified gradient system (11) that effected a more marked separation between putrescine (81 min) and *S*-adenosylmethionine (45 min).

Isolation and characterization of strains having an increased activity of ornithine decarboxylase. Crude extracts of most laboratory strains of *S. cerevisiae* contain barely detectable levels of ornithine decarboxylase when grown on YPAD, a rich medium that contains polyamines. Even though it has been shown (D. G. Kay, R. A. Singer, and G. C. Johnston, 1979 Cold Spring Harbor Meet. Mol. Biol. Yeast, p. 41) that much more ornithine decarboxylase could be obtained in extracts of cells grown in a minimal medium to a low cell density (Table 2), it was desirable to use crude media for the mutagenesis experiments, since mutagenized cells grow poorly in purified media. We therefore first selected mutants that had high concentrations of ornithine decarboxylase in extracts of cells, even when grown on YPAD. For this purpose we mutagenized strain AN33, which is wild type with respect to polyamine metabolism. A strain (J25) with over 100-fold-increased ornithine decarboxylase activity was found (Table 2). Further mutagenesis of J25 produced a strain with a higher ornithine decarboxylase activity (68-C).

RESULTS

Isolation and phenotypic characterization of strains which do not make putrescine. After mutagenesis of wild-type strain AN33, we found a mutant (M57) by method B which lacked ornithine decarboxylase and was not able to grow at normal rates on H medium unless polyamines were added (e.g., 10^{-5} M pu-

TABLE 2. *Levels of polyamine biosynthetic enzymes in crude extracts of various strains*

Strain	Genotype	Sp act (pmol/min per mg of protein) of:				
		Ornithine decarboxylase[a]		*S*-adenosylmethionine decarboxylase[b]	Putrescine aminopropyltransferase[b]	Spermidine aminopropyltransferase[b]
		YPAD	H medium			
AN33	*spe10*$^+$	0.023	13	13	1.0	0.073
J25	*spe10*$^+$	2.7	25	13	1.5	0.058
68C	*spe10*$^+$	27.8	ND[c]	ND[c]	ND[c]	ND[c]
73-7C	*spe10-1*	<0.01	<0.01	14	0.7	0.050
81-1C	*spe10-2*	<0.01	<0.01	16	0.6	0.079
82-1B	*spe10-3*	<0.01	ND[d]	14	1.2	0.099
73-7CB	*spe10-1* *spe40-1*	3.9	150	ND[e]	1.6	<0.001
81-1CB	*spe10-2* *spe40-2*	1.7	100	ND[e]	0.7	<0.001
82-1BB	*spe10-3* *spe4-1*	<0.01	<0.01	ND[c]	0.7	<0.001

[a] Cultures were grown at 22°C to an absorbance at 650 nm of 1 to 2 on YPAD (a polyamine-rich medium) or to an absorbance of 0.2 on H medium (a polyamine-free medium) at pH 7.

[b] Cultures were grown at 22°C to an absorbance at 650 nm of 1 to 2 on YPAD.

[c] ND, Not determined.

[d] ND, Not determined, as this strain does not grow on H medium.

[e] ND, Not determined, but this enzyme was present since spermidine is synthesized in these strains.

trescine, spermidine, or spermine). We designated the gene in which this mutation occurs *spe10* (and this strain *spe10-1*) because *spe10* is probably not the structural gene for ornithine decarboxylase (see below).

Seven additional mutants with no detectable ornithine decarboxylase were obtained from the "overproducing" strains described above. As with mutant M57 (see above) all of these mutants were unable to grow at a normal rate on H medium unless polyamines were added. All eight mutants were in the same complementation group (determined by complementation and allelism tests), but there were a few cases of complementing alleles. For example, strain 6A-30 complemented strain 81-5C, but neither strain complemented most of the other ornithine decarboxylase-deficient strains, and the mutation in strain 6A-30 was inseparable from the mutation in strain 81-5C in crosses. It is important to emphasize that the phenotype exhibited by a *spe10* mutant segregates 2:2 in crosses with either wild type or strains that overproduce ornithine decarboxylase, indicating that the background that causes an overproduction of ornithine decarboxylase has no effect on the *spe10* mutant phenotype.

spe10 is not centromere linked or linked to any of the other markers tested. *spe10* mutants complement mutants defective in putrescine aminopropyltransferase (*spe3*) (this enzyme is different from spermidine aminopropyltransferase [unpublished data]) and mutants defective in *S*-adenosylmethionine decarboxylase (*spe2*) (2).

Three of these polyamine-requiring mutants, *spe10-1* (M57), *spe10-2* (K14), and *spe10-3* (M4), were each crossed with strain 53 (**a** *ade1*) to remove possible mutations in other loci. In the progeny we found cosegregation of the absence of ornithine decarboxylase, slow growth, and stimulation by polyamines; three haploid strains from these crosses, 73-7C, 81-1C, and 82-1B, had 29-h, 64-h, and infinite doubling times, respectively, in H medium at 26°C in the absence of added polyamines. After the *spe10-3* strain (82-1B) was depleted of endogenous polyamines, there was absolutely no growth of this strain during a 2-week period either after streaking for single colonies on solid polyamine-deficient H medium or after inoculating liquid polyamine-deficient H medium. Analyses of *spe10-1* and *spe10-2* strains grown in H medium showed no putrescine, spermidine, or spermine (Table 3), although it is possible that undetectable levels were present. The addition of 10^{-6} M spermidine (or 10^{-5} M putrescine or spermine) to any of these strains in H medium at 26°C restored the doubling time to the doubling time of the wild-type strain (AN33) (3 h). The addition of any polyamine at a concentration of as little as 10^{-10} M to H medium allowed very slow growth of a *spe10-3* strain; however, no polyamines were detected if a *spe10-3* strain grown with this low concentration of amines was analyzed for polyamines (i.e., the very small concentrations of polyamines that allowed growth could not be detected by our assay). All *spe10* mutants had normal levels of *S*-adenosylmethionine decarboxylase, putrescine aminopropyltransferase, and spermidine aminopropyltransferase (Table 2).

TABLE 3. *Levels of polyamines in various strains*[a]

Strain	Genotype	Concn (nmol/mg [wet wt] of yeast) of:		
		Putrescine	Spermidine	Spermine
AN33	Wild type	0.45	2.0	0.30
J25	Unknown	0.32	0.48	0.03
68-C	Unknown	0.26	1.8	0.06
73-7C	*spe10-1*	<0.001	<0.0005	<0.001
81-1C	*spe10-2*	<0.001	<0.0005	<0.001
82-1B	*spe10-3*	ND[b]	ND	ND
73-7CB	*spe10-1 spe40-1*	0.01	0.12	<0.001
81-1CB	*spe10-2 spe40-2*	0.03	0.4	<0.001
82-1BB	*spe10-3 spe4-1*	<0.001	<0.0005	<0.001
86-9C	*spe4-1*	0.32	1.0	<0.001

[a] Grown in H medium (pH 7) to an absorbance at 650 nm of approximately 1.

[b] ND, Not determined, since this strain does not grow in H medium.

We have shown previously (2, 3) that the ability to sporulate and the ability to maintain the killer plasmid require spermidine or spermine; i.e., these processes do not occur in *spe2* mutants that do not contain these amines but do contain putrescine. To determine whether putrescine is also necessary, we tested sporulation and the maintenance of the killer plasmid in *spe10* mutants. A *spe10-3* diploid (85-5B × 85-7C) from rich medium was streaked onto either polyamine-free sporulation medium (enough amines remained for very poor growth) or the same medium containing 10^{-4} M spermidine or spermine. After 6 weeks at 26°C, no asci were observed in the absence of added spermidine or spermine. Normal sporulation occurred if either of these amines was present in the sporulation medium (i.e., without any putrescine). Since sporulation medium itself markedly limits growth because of the absence of a sufficient nitrogen source, it does not seem likely that restriction of growth due to the absence of amines was a significant factor in preventing sporulation in the deficient culture.

To test for the ability to maintain the killer plasmid in *spe10* strains, the wild-type killer

strain A364A (K^+ R^+; able to kill sensitive cells and resistant to the killer toxin) was crossed with a *spe10-3* K^- R^- strain (85-7C). The 12 tetrads from this cross exhibited 2:2 segregation for *spe10-3* and $4^+:0^-$ segregation on YPAD for the ability to kill a sensitive (K^- R^-) strain (5 × 47) on MB medium at 20°C. When transferred from this rich medium to polyamine-deficient medium, the segregants containing *spe10-3*, which barely grew as the internal amine levels were depleted, lost the ability to kill a sensitive strain when examined after 2 days on the deficient medium (the ability to kill was not restored by subsequent addition of polyamines). However, when grown in the presence of 10^{-4} M spermidine or spermine (i.e., without putrescine), *spe10-3* segregants maintained the ability to kill sensitive strains. Thus, spermidine or spermine is sufficient to maintain the killer plasmid; putrescine is not necessary. In control experiments *spe10*$^+$ segregants were able to maintain the killer plasmid in the absence or presence of these amines.

Bypass of the defect in *spe10* mutants. Prolonged incubation of organisms containing a *spe10* mutation in the absence of added polyamines usually resulted in the selection of strains which outgrew the *spe10* mutant culture (rare isolated colonies appeared through a lawn of the almost completely depleted *spe10* mutant on solid H medium). These strains contained mutations in either of two different genes, which were designated *spe4* and *spe40*. Each of these mutations segregated 2:2 in a *spe10* mutant background. In all *spe4* or *spe40* mutants isolated and in all crosses involving them, the phenotype of these bypass mutations was associated with the inability to synthesize spermine from spermidine, i.e., a defect in the activity of spermidine aminopropyltransferase (spermine synthase), the fourth enzyme in the polyamine biosynthetic pathway (Fig. 1).

The *spe40* mutation was found in both *spe10-1* and *spe10-2* backgrounds. Strains of the genotypes *spe10-1 spe40-1* (e.g., 73-7CB) and *spe10-2 spe40-2* (e.g., 81-1CB) have the following properties. (i) At 26°C, the growth rate without polyamine addition was the same as the rate with polyamine addition, about 3.6 h (only slightly slower than that of a wild-type strain). (ii) There was synthesis of small amounts of putrescine and spermidine, but no spermine (Table 3). (iii) High levels of ornithine decarboxylase were observed (Table 2). (iv) There was no detectable spermidine aminopropyltransferase (spermine synthase) in crude extracts, and no spermine (<0.001 nmol/mg [wet weight]) was detected by amine analysis of cells grown at 22°C to an absorbance at 650 nm of 1 on H medium to

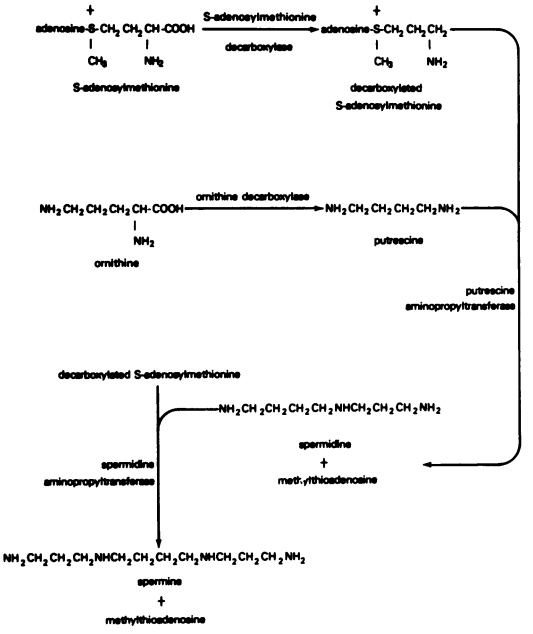

FIG. 1. *Pathway for the biosynthesis of polyamines in yeast.*

which 10^{-4} M spermidine had been added. The parent strains, *spe10-1* (73-7C) and *spe10-2* (81-1C), produced spermidine aminopropyltransferase. All strains produced putrescine aminopropyltransferase (Table 2). (v) The *spe40* mutation was phenotypically dominant; i.e., a diploid made from the cross *spe10-2 spe40-2* × *spe10-2* (e.g., 81-1CB × 81-12A) grew in the absence of polyamines. (vi) A cross of a *spe10-1 spe40-1* strain with a wild-type strain (e.g., 73-7CB × 200) and subsequent sporulation resulted in the appearance of only rare polyamine-requiring spore clones (2 clones of 24 tetrads examined), and in the case of a *spe10-2 spe40-2* strain crossed with a wild-type strain (e.g., 81-1CB × 200), no polyamine-requiring spore clone was detected (of 12 tetrads tested); i.e., *spe40* is tightly linked to *spe10*. Because *spe40-1* and *spe40-2* are dominant, they cannot be shown to map in the same gene by complementation testing.

spe4 was found initially in a *spe10-3* background. Strains of the genotype *spe10-3 spe4-1* (e.g., 82-1BB) had the following properties. (i) At 26°C the doubling time without polyamine addition was approximately 10 h, which was reduced to approximately 4 h upon polyamine addition. (ii) There was no detectable putrescine, spermidine, or spermine (Table 3). (iii) Ornithine decarboxylase could not be detected (Table 2). (iv) There was no detectable spermidine aminopropyltransferase, and no spermine

(<0.001 nmol/mg [wet weight]) was detected by amine analysis of cells grown at 22°C to an absorbance at 650 nm of 1 on H medium to which 10^{-4} M spermidine had been added. The parent strain, with a *spe10-3* genotype (e.g., 82-1B), produced spermidine aminopropyltransferase, and both strains produced putrescine aminopropyltransferase (Table 2). (v) The *spe4-1* mutation was phenotypically recessive; a diploid made from the cross *spe10-3 spe4-1* × *spe10-3* (e.g., 82-1BB × 85-5B) had all of the phenotypes of a strain containing only *spe10-3*. (vi) A cross of a *spe10-3 spe4-1* strain with a wild-type strain (e.g., 82-1BB × 53) and sporulation resulted in the appearance of spore clones of the *spe10-3* genotype (i.e., not containing *spe4-1*) approximately 25% of the time (i.e., *spe4-1* is not linked to *spe10-3*) (Table 4).

The properties of the mutations in *spe4* and *spe40* were not attributable to the different *spe10* mutant allelic backgrounds in which they were isolated. When growth was measured in the absence of amines in suitable diploids, the *spe4* mutation was always phenotypically recessive, and the *spe40* mutation was always phenotypically dominant regardless of the *spe10* allelic background of the diploid (Table 5). Further proof that a mutation in *spe4* caused the phenotype described above was obtained from the transfer of the *spe4-1* mutation from a *spe10-3 spe4-1* parent (82-1BB) to a *spe10*$^+$ background. Of the spore clones from the cross of *spe10-3 spe4-1* with a wild-type strain (82-1BB × 53), those that were *spe10*$^+$ (i.e., no effect of added polyamines on growth) were examined for polyamine content. Although the parent *spe10-3 spe4-1* strain (82-1BB) had undetectable levels of putrescine and spermidine, all of these spore clones had near wild-type levels of putrescine and spermidine, but some had no spermine, the phenotype of a mutation in *spe4-1* (e.g., 86-9C) (Table 3). One such *spe4-1* clone (86-10C) was crossed with a *spe10-3* strain (105-7), and after sporulation spore clones of the genotype *spe10-3 spe4-1* were observed (Table 4). Another *spe4-1* strain, 86-9C (which was demonstrated to be *spe10*$^+$ since all of the spore clones from a cross [12 tetrads] of 86-9C with a wild-type strain [200] had no polyamine requirement), was then crossed with a *spe10-1* strain (75-19A), and after sporulation suppression of *spe10-1* was observed in the *spe10-1 spe4-1* segregants (Table 4). These strains had properties identical to those of strains of the genotype *spe10-3 spe4-1* (i.e., the recessive behavior of *spe4-1* [Table 5] and a faster doubling time in the presence of polyamines than in their absence), even though *spe4-1* was in a different *spe10* mutant background (*spe10-1*) than the one in which it was isolated (*spe10-3*).

TABLE 4. *Segregation of spe4-1 and spe10 mutations*[a]

Cross	No. of:		
	Parental ditype	Nonparental ditype	Tetratype
spe10-3 spe4-1 × wild type (82-1BB × 53)	2	1	9
spe4-1 × *spe10-3* (86-10C × 105-7)	1	2	6
spe4-1 × *spe10-1* (86-9C × 75-19A)	2	3	7

[a] The four possible combinations of the two genes cause the following effects on growth (observed after 2 days) in the absence of spermidine: *spe10 spe4*$^+$, no growth; *spe10 spe4*, poor growth; *spe10*$^+$ *spe4* and *spe10*$^+$ *spe4*$^+$, normal growth. The latter two can be distinguished by analysis for spermine; spermine is absent in the *spe10*$^+$ *spe4* mutant. Growth was normal in all cases after the addition of 10^{-5} M spermidine.

We show above that *spe40* is linked to *spe10* and that *spe4* is not linked to *spe10*. To demonstrate further that *spe40* and *spe4* are not linked, the following crosses were made: *spe10-1 spe4-1* × *spe10-1 spe40-1* (127-4D × 73-7CB) and *spe10-1 spe4-1* × *spe10-2 spe40-2* (127-4D × 81-1CB). In both cases, tetratypes (after sporulation) of the following types were observed: two spore clones with no polyamine requirement for growth (*spe10 spe40-1* [or *spe40-2*] and *spe10 spe40-1* [or *spe40-2*] *spe4-1*), one spore clone with a 10-h doubling time without polyamines (*spe10 spe4-1*), and one spore clone with a 30-h doubling time without polyamines (i.e., *spe10*) (Table 6). Therefore, even though a mutation in either *spe4* or *spe40* causes a spermidine aminopropyltransferase deficiency, these two genes are not linked. It is not known whether either *spe4* or *spe40* is the structural gene for spermidine aminopropyltransferase.

The finding that the absence of spermine partially relieves the deficiency of ornithine decarboxylase in *spe10* mutants suggests that spermine or spermidine aminopropyltransferase (spermine synthase) plays a role in the regulation of the biosynthesis of ornithine decarboxylase in yeast. However, the addition of spermine or spermidine during growth of the bypassed *spe10* strains led to confusing results. As Table 7 shows, although the addition of spermine markedly decreased the ornithine decarboxylase levels in both a *spe10-2 spe40-2* strain (81-1CB) and a *spe10-1 spe40-1* strain (73-7CB), the effect was more pronounced in the former strain. In addition, spermidine also decreased the ornithine decarboxylase levels in these strains. Furthermore, in all cases the residual ornithine de-

TABLE 5. *Characteristics of bypass mutations in heteroallelic spe10 backgrounds*

Diploid	Relevant genotypes	Growth in absence of polyamines[a]	Dominance or recessiveness of bypass mutation[b]
73-7CB × 75-19A	*spe10-1 spe40-1* × *spe10-1*	+	Dominant
73-7CB × 81-12A	*spe10-1 spe40-1* × *spe10-2*	+	Dominant
73-7CB × 85-6A	*spe10-1 spe40-1* × *spe10-3*	+	Dominant
81-1CB × 75-16A	*spe10-2 spe40-2* × *spe10-1*	+	Dominant
81-1CB × 81-12A	*spe10-2 spe40-2* × *spe10-2*	+	Dominant
81-1CB × 87-4A	*spe10-2 spe40-2* × *spe10-3*	+	Dominant
82-1BB × 75-16A	*spe10-3 spe4-1* × *spe10-1*	−	Recessive
82-1BB × 81-5C	*spe10-3 spe4-1* × *spe10-2*	−	Recessive
82-1BB × 85-5B	*spe10-3 spe4-1* × *spe10-3*	−	Recessive
127-2B × 75-19A[c]	*spe10-1 spe4-1* × *spe10-1*	−	Recessive
127-2B × 81-5C[c]	*spe10-1 spe4-1* × *spe10-2*	−	Recessive
127-2B × 85-6A[c]	*spe10-1 spe4-1* × *spe10-3*	−	Recessive

[a] Growth was scored on solid H medium after 2 days. +, Growth of any sort (a *spe40* mutation, if dominant, would give good growth, and a *spe4* mutation, if dominant, would give poor growth); −, no growth (both *spe40* and *spe4* mutations, if recessive, would give this phenotype).

[b] In a diploid heterozygous for the bypass (a mutation in *spe4* or *spe40*) and homozygous for the *spe10* mutation.

[c] Strain 127-2B was isolated after sporulation of the diploid strain 86-9C × 75-19A (Table 4).

TABLE 6. *Segregation of spe4 and spe40 mutations*[a]

Cross	No. of:		
	Parental ditype	Nonparental ditype	Tetratype
spe10-1 spe4-1 × *spe10-1 spe40-1* (127-4D × 73-7CB)	1	2	5
spe10-1 spe4-1 × *spe10-2 spe40-2* (127-4D × 81-1CB)	2	2	7

[a] The four possible combinations (*spe10* is always present) cause the following effects on growth (observed after 2 days) in the absence of spermidine: *spe4 spe40* and *spe4*$^+$ *spe40*, normal growth; *spe4 spe40*$^+$, poor growth; *spe4*$^+$ *spe40*$^+$, no growth. Growth was normal in all cases after the addition of 10^{-5} M spermidine.

carboxylase activity was higher than that observed in *spe10* mutants. Therefore, since the addition of spermine does not result in undetectable levels of ornithine decarboxylase, the increase in ornithine decarboxylase activity in *spe4* and *spe40* mutants is not explained simply by the removal of spermine from the cells.

DISCUSSION

A *spe10-3* mutant, which lacks ornithine decarboxylase, is completely unable to grow in polyamine-deficient medium. Thus, polyamines are absolutely necessary for the growth of yeast. As little as 10^{-10} M spermidine allows growth (but at a slower rate), and we found that all cells in a culture growing exponentially at this concentration were viable. This means that if all of the spermidine in the medium were concentrated intracellularly, a culture at an absorbance at 650 nm of 1 (2×10^7 cells per ml) would have a maximum of 100 pmol of polyamines per g (wet weight) of yeast, which is 20,000-fold less than the concentration in wild-type cells (2 μmol/g). Therefore, it is probable that at least one function of polyamines is catalytic and not structural. We want to emphasize the low level of spermidine that gives a phenotypic response, since such levels would be below the limit of detection of our analytical procedures for amines.

It is probable that the most important function of putrescine in yeast is to serve as a precursor for spermidine and spermine biosyntheses, since strains containing the *spe10* mutation appear normal in all aspects tested when cultures are supplemented with only spermidine or spermine, and *spe2* strains which lack spermidine and spermine, but not putrescine (2), grow poorly. Putrescine is not necessary for sporulation and the maintenance of the killer plasmid, nor does it satisfy the requirement for spermine or spermidine.

Three types of mutations which affect ornithine decarboxylase activity in yeast have been found.

(i) Increased levels of ornithine decarboxylase have been found in strains such as J25 and 68-C, even when the cells are grown to high cell densities or in polyamine-containing media.

(ii) Complete loss of ornithine decarboxylase activity has been found in three *spe10* mutants.

(iii) A third class of mutants are the bypass

TABLE 7. *Effect of polyamine addition to the growth medium on ornithine decarboxylase in spe10 mutants containing a spe4 or spe40 mutation*

Strain	Genotype	Growth medium[a]	Ornithine decarboxylase activity (pmol/min per mg)
73-7C	*spe10-1*	H	<0.01
73-7CB	*spe10-1 spe40-1*	H	150
		H + 10^{-4} M spermidine	4.2
		H + 10^{-4} M spermine	17
81-1C	*spe10-2*	H	<0.01
81-1CB	*spe10-2 spe40-2*	H	100
		H + 10^{-4} M spermidine	1.8
		H + 10^{-4} M spermine	1.7
82-1B	*spe10-3*	H	ND[b]
82-1BB	*spe10-3 spe4-1*	H	<0.01
		H + 10^{-4} M spermidine	<0.01
		H + 10^{-4} M spermine	<0.01

[a] All growth was at 22°C.
[b] ND, Not determined, since this strain does not grow in H medium.

mutants (*spe4* and *spe40*), which arose from *spe10* mutants and permit *spe10* mutants to grow in the absence of added polyamines. Both *spe4* and *spe40* mutants have no spermidine aminopropyltransferase, even though the parent *spe10* mutant strains do have this activity. Therefore, the bypass mutants are not able to synthesize spermine, even if spermidine is added to the growth medium. The *spe4* mutation is not linked to the *spe10* mutation. *spe10 spe4* mutants do not have any detectable ornithine decarboxylase or amines. It is hard to interpret this lack of amines, however, since, as indicated above, undetectable levels of amines are sufficient to permit slow growth. The *spe40* mutation is tightly linked to the *spe10* mutation. *spe10 spe40* mutants do have high ornithine decarboxylase levels and contain both putrescine and spermidine.

Since the *spe10* mutation can be bypassed by either of two mutations (*spe4* or *spe40*) that result in the simultaneous loss of another enzyme (spermidine aminopropyltransferase), we postulate that *spe10* is not the structural gene for ornithine decarboxylase (i.e., that it is a regulatory gene). This postulation is based only on indirect evidence, however. Only by complex and unlikely situations, such as the presence of two structural genes coding for ornithine decarboxylase, could one postulate that the *spe10* gene is the structural gene. It should be kept in mind, however, that no mutation resulting in the loss of ornithine decarboxylase and mapping at a location other than *spe10* has been found despite exhaustive searches.

The finding of a relatively complex system for the synthesis and regulation of ornithine decarboxylase and polyamine biosynthesis in yeast is of particular importance because of the widespread interest in the control of ornithine decarboxylase in mammalian systems under conditions that stimulate growth. Yeast, a eucaryote, may be the most suitable system in which to study this control, as desired genotypes can be easily obtained and manipulated.

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LITERATURE CITED

1. **Bowman, W. H., C. W. Tabor, and H. Tabor.** 1973. Spermidine biosynthesis: purification and properties of propylamine transferase from *Escherichia coli*. J. Biol. Chem. **248**:2480–2486.
2. **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.
3. **Cohn, M. S., C. W. Tabor, H. Tabor, and R. B. Wickner.** 1978. Spermidine or spermine requirement for killer double-stranded RNA plasmid replication in yeast. J. Biol. Chem. **253**:5225–5227.
4. **Hafner, E. W., C. W. Tabor, and H. Tabor.** 1979. Mutants of *Escherichia coli* that do not contain 1,4-diaminobutane (putrescine) or spermidine. J. Biol. Chem. **254**:12419–12426.
5. **Kee, S. G., and J. E. Haber.** 1975. Cell cycle-dependent induction of mutations along a yeast chromosome. Proc. Natl. Acad. Sci. U.S.A. **72**:1179–1183.
6. **Mortimer, R. K., and D. C. Hawthorne.** 1973. Genetic mapping in *Saccharomyces*. IV. Mapping of temperature-sensitive genes and use of disomic strains in localizing genes. Genetics **74**:33–54.
7. **Pegg, A. E., and H. G. Williams-Ashman.** 1968. Biosynthesis of putrescine in the prostate gland of the rat. Biochem. J. **108**:533–539.
8. **Tabor, C. W., and H. Tabor.** 1976. 1,4-Diaminobutane (putrescine), spermidine, and spermine. Annu. Rev. Biochem. **45**:285–306.
9. **Tabor, C. W., H. Tabor, and E. W. Hafner.** 1978.

Escherichia coli mutants completely deficient in adenosylmethionine decarboxylase and in spermidine biosynthesis. J. Biol. Chem. **253**:3671–3676.

10. **Tabor, H., S. M. Rosenthal, and C. W. Tabor.** 1958. The biosynthesis of spermidine and spermine from putrescine and methionine. J. Biol. Chem. **233**:907–914.
11. **Tabor, H., and C. W. Tabor.** 1975. Isolation, characterization, and turnover of glutathionylspermidine from *Escherichia coli*. J. Biol. Chem. **250**:2648–2654.
12. **Tabor, H., C. W. Tabor, and E. W. Hafner.** 1976. Convenient method for detecting $^{14}CO_2$ in multiple samples: application to rapid screening for mutants. J. Bacteriol. **128**:485–486.
13. **Whitney, P. A., and D. R. Morris.** 1978. Polyamine auxotrophs of *Saccharomyces cerevisiae*. J. Bacteriol. **134**:214–220.