Letter to the Editor

The Yeast *HSM3* Gene Is Involved in DNA Mismatch Repair in Slowly Dividing Cells

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PREVIOUSLY, we reported some properties of *Saccharomyces cerevisiae* mutants bearing the *hsm3* mutation (Fedorova *et al.* 1998). On the basis of our data we concluded that the *HSM3* gene controls a minor mismatch repair pathway that is different from the repair pathway controlled by *MSH2*. Based on our work Merker *et al.* (1999) constructed a null mutation in the *S. cerevisiae HSM3* gene. Using our and their strains bearing *hsm3* deletions, Merker *et al.* did not observe an increase in the rate of spontaneous mutation to canavanine resistance, but they confirmed our data showing increased UV-induced mutagenesis in an *hsm3* mutant.

To resolve the differences in the results obtained in the two laboratories, we carefully analyzed possible differences in the methods used for the measurement of the spontaneous mutation rates and we measured the spontaneous mutation rate in their and our *hms3* strains, using our methods.

Merker *et al.* used a richer selective medium, containing very high canavanine concentrations (60–120 mg/liter), a higher number of cells plated on the selective medium (5×10^8), and a shorter period of yeast cell growth on the selective medium. Under these conditions canavanine-resistant cells can rapidly divide and form colonies in 3 days on a selective medium containing a high concentration of canavanine that greatly reduces or eliminates residual growth of canavanine-sensitive cells. So the authors count only the mutations that arise during growth in nonselective medium before plating on the selective medium.

We used the method of ordered plating (von Borstel 1978) to measure spontaneous mutation rates. The tested yeast cultures were grown on plates with complete medium for 1 day. Then, 5 ml of a suspension (10^6 cells/ml) was prepared. A special 150-stamp replicator was dipped into this suspension and inverted on a plate with selective medium containing 15–60 mg/liter canavanine and 5% liquid YEPD. The replicator places 150 equal drops of yeast suspension (about 2 μl each; ~2000 cells) at equal distances from each other. The mutants have faster growth that shows up as papillae on the spots with limited growth of the tested culture. After 14–15 days of incubation the papillae and the total viable cells were counted. The latter was done after washing the cells from the entire plate or from a number of single-drop replicas lacking visible papillae. The yeast grow uniformly when plated in this manner. This minimizes the variation of frequencies (von Borstel 1978). If the papillae arose within 3 days after inoculum plating and had the same size, we concluded that the mutant cells preexisted in the culture before we plated them and these experiments were ignored. The rates of mutation per cell division were determined by dividing the number of papillae by the total number of viable cells on the plate.

In these experiments we used a lower concentration of canavanine (15–60 mg/liter) that allows 8–10 cell divisions and the mutations can arise during the period of cell growth on selective media. Our selective media limit the yeast cell growth, and we measure the spontaneous mutation rates in slowly dividing cells. The results obtained show that *hsm3* deletions in all strains considerably increase (five- to ninefold) the spontaneous mutation rate in comparison with the wild-type strains. These differences in the methods of the spontaneous mutation rate measurement may explain the differences in the results presented in Table 1.

In the fluctuation method of the median we used a higher concentration of canavanine (60–120 mg/liter) and a high cell concentration (2×10^8 per plate), and the results were counted after 3 days of growth on our selective media. In these experiments we did not see canavanine-resistant colonies in any strain (Table 1). This fact can be explained by slow growth of canavanine-resistant colonies on the poor medium with high cana-

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TABLE 1

Spontaneous mutation rates to canavanine resistance measured by different methods

		Spontaneous mutation rates $\times 10^{-7}$		
			Fluctuation test of the median in 3 days of growth	
Strain[a]	Pertinent genotype	The method of ordered plating[b]	Our experiments	Merker *et al.*'s experiments[c]
11D-3031	Wild type	3.6 (40)[d]	<0.1 (120)	5.8 (120)
2LMG-316	*hsm3*Δ	32.0 (40)	<0.1 (120)	9.3 (120)
MS71	Wild type	2.7 (15)	<0.1 (60)	3.2 (60)
JDM7	*hsm3*Δ	18.1 (15)	<0.1 (60)	2.6 (60)
JDM8	*hsm3*Δ	14.2 (15)	<0.1 (60)	2.8 (60)

[a] Strains 11D-3031 and 2LMG-316 are described by Fedorova *et al.* (1998). The others are described by Merker *et al.* (1999).

[b] Our selective medium: 2 g KH_2PO_4; 1 g $MgSO_4$; 1 g $(NH_4)_2SO_4$; 20 g glucose; vitamins; 15 g Bacto agar; 1 liter water; canavanine (see footnote d); 5% liquid YEPD medium.

[c] The selective medium used by Merker *et al.*: 1.7 g yeast nitrogen base; 5 g ammonium sulfate; 20 g dextrose; 30 g agar; adenine, aspartic acid, histidine, leucine, lysine, methionine, phenylalanine, tryptophan, tyrosine, uracil, isoleucine, glutamic acid, valine, threonine, and serine; 1 liter water; canavanine (see footnote d).

[d] Canavanine concentrations in micrograms per milliliter.

vanine concentrations. After 6–7 days we observed the appearance of rare canavanine-resistant colonies in all strains (data not shown).

The analysis of these results allowed us to conclude that the *hsm3* mutation increases the spontaneous mutation rate only during slow cell growth and that this mutation does not influence this process in rapidly dividing cells. It is possible that repair processes connected with recombination are less effective in rapidly growing cells, in which replication and cell division occur very rapidly. We suggest that the product of the *HSM3* gene acts in the correction of DNA heteroduplexes arising during recombination (S. V. Kovaltzova, I. V. Fedorova, L. M. Gracheva, T. A. Evstuhina and V. G. Korolev, unpublished data). This may explain why the mutator effect of *hsm3* mutations was not observed in rapidly dividing cells.

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