Rem1 has no observable effect on intragenic meiotic recombination frequency in *S. Pombe*

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INTRODUCTION

In this study, we attempted to duplicate the reported three to four-fold decrease in meiotic intragenic recombination associated with *rem1* (deletion with *kanR* PCR) mutants (Ayté et al.). The purposes of this study were to investigate whether the reported behavior could be observed among newly constructed *rem1* strains, and to provide context for tetrad analysis. By calculating gross intragenic recombinant frequencies in *ade6* from *rem1* homozygous matings, and finding no significant difference from wild-type controls, we conclude that there is no observable hypo-rec activity of *rem1*.

MATERIALS AND METHODS

Strains and Media The strains used in this study are listed in Table 1. Strains prefixed with JA were transported internationally in papers and immediately bled into YEL supplemented with adenine for culture at 32° C. All other strains were streaked onto YEA4S at room temperature from cryogenic storage at 80°C and subsequently cultured. Strains that were *tps* mutants were cultured, colonized, and mated at 25°C. Nutrients were added in 2 mg per plate.

Meiosis To induce meiosis in the haploid strains, parental cultures were freshly inoculated at 1:20 in YEL-ade. Once saturated, 50 µL of each culture was mixed and centrifuge-washed twice with water. Nitrogen starvation was achieved by spotting the mixture onto SPA supplemented with all required amino acids, purines, and pyrimidines. Meiosis was allowed to proceed from two to four days. Verification of mating was performed by identification of asci under wet-mount. The cell/ascus spot was then treated for 24 hours with a 1:200 dilution of glusulase in 0.5 mL water to digest the membranes of the asci. To eliminate usporulated cells, 0.5 mL of 60% ethanol was added to the resulting spores for 15 – 20 min. at room temperature. After this treatment, the spores were centrifuge-washed twice, and stored with high retention of viability.

Selection of Recombinants Spores from the crosses listed in Table 2 were plated at appropriate dilutions on YEA4S, to allow expression of *ade6-M26* and *ade6-52* parental phenotypes, and YEA4S+G (guanine) to inhibit growth of adenine auxotrophs and select for recombinants. Plates were incubated at 32°C until colonies were visible.

TABLE 1. Strains used in the study

Strain	Genotype
GP 13	*h^- ade6-52*
GP 24	*h^+ ade6-M26*
GP 488	*h^+ ade6-M26 arg1-230*
GP 720	*h^- ura4-294 ade6-52 arg1-230 leu1-32*
GP 2664	*h^- ade6-52 arg1-230 leu1-32*
GP 4917	*h^+ ura4-D18 ura4-aI ade6-52 tps16*
GP 5674	*h^+ ura4-D18 ade6-3049*
JA 427	*h^+ rem1::kn ade6-M26 arg1-230*
JA 456	*h^-.rem1:kn ura4-D18 arg1-230*
JA 493	*h^+ rem1::kn ura4-294 ade6-52 leu1-32*

RESULTS

TABLE 2. List of crosses. Dilution X.Y indicates plating of $\frac{Y}{10}$ mL of 10^{-X} dilution of original titer.

Cross	Strains Crossed	Diploid genotype at *rem1*	Dilution on YEA4S	ade6-M26	ade6-52	ade+	Dilution on YEA4S+G	ade+	Recombinants per million
1	*GP 13 GP 24*	*+/+*	*4.2*	*60*	*62*	*-*	*2.2*	*129*	*5200*
2	*JA 427 JA 493*	*-/-*	*4.2*	*44*	*56*	*-*	*2.2*	*48*	*2400*
	Replated		*3.1*	*211*	*187*	*-*	*1.2*	*238*	*2900*
3	*JA 427 JA 493*	*-/-*	*3.2*	*194*	*150*	*-*	*1.4*	*182*	*2600*
6	*GP 488 GP 2664*	*+/+*	*3.2*	*125*	*85*	*-*	*1.4*	*84*	*2000*
7-1	*JA 427 JA 493*	*-/-*	*3.1*	*300*	*225*	*3*	*1.2*	*772*	*7300*
7-2	*JA 427 JA 493*	*-/-*	*3.1*	*263*	*250*	*4*	*1.2*	*610*	*5900*
7-3	*JA 427 JA 493*	*-/-*	*3.1*	*180*	*208*	*2*	*1.2*	*558*	*7100*
7-4	*JA 427 JA 493*	*-/-*	*3.1*	*228*	*220*	*0*	*1.2*	*445*	*4900*
8-1	*GP 488 GP 720*	*+/+*	*3.1*	*285*	*257*	*2*	*1.2*	*692*	*6000*
8-2	*GP 488 GP 720*	*+/+*	*3.1*	*112*	*128*	*0*	*1.2*	*338*	*7000*
8-3	*GP 488 GP 720*	*+/+*	*3.1*	*150*	*182*	*2*	*1.2*	*262*	*3900*
8-4	*GP 488 GP 720*	*+/+*	*3.1*	*168*	*194*	*2*	*1.2*	*426*	*5800*

Crosses 7-1 through 8-4 where performed in parallel as isogenic quadruplets to isolate the effects of *rem1*. The crosses, segregant constitution, and recombinant frequencies are listed in Table 2. The recombinant frequency, computed for isogenic crosses, (per 10^6), for mutants was 6300 ± 560 (standard error of the mean) and 5600 ± 650 for w.t. We verified that the parental strains were *rem1*, by plating 50 random colonies and replicating onto 4S containing G418. We did not perform a PCR to determine the sequence of the substitution.

Differences in the protocols used for synchronous meiosis and/or selection of recombinants are quite possibly the source of irreproducibility in our lab. Thus, we would appreciate clarification about how crosses were performed and how recombinant frequency was calculated.

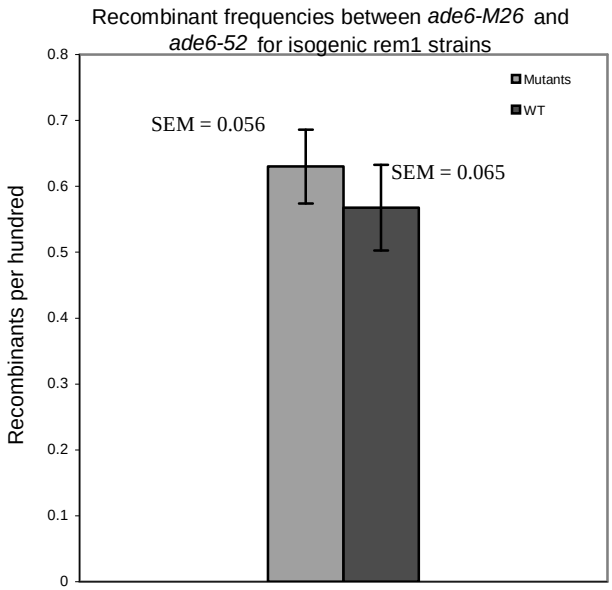

FIGURE 1. Recombinant frequencies for quadruplicate crosses in mutant and w.t. backgrounds.