GENE CONVERSION AND INTRAGENIC RECOMBINATION AT AT THE *SUP6* LOCUS AND THE SURROUNDING REGION IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Spontaneous secondary mutations of the ochre suppressor *SUP6* were selected in a haploid strain of *Saccharomyces cerevisiae*. Unselected tetrads were dissected from crosses heterozygous for one of three alleles of *SUP6* and for three other loci in this region which span a length of 14 map units (*his2*, *cdc14* and *met10*). The study showed that all of these markers were characterized by high frequency of meiotic gene conversion and long conversion lengths which frequently extended into adjacent marked loci. Despite the high conversion frequency of *SUP6*, recombination between alleles of this locus reached a maximum frequency of only 2×10^{-3} prototrophs/spore. Although the allelic recombination frequencies were not distance dependent and consequently could not be used to order the alleles, the inequality between the two recombinant outside marker combinations among selected intragenic recombinants produced an internally consistent map of the suppressor locus. Recombination at *SUP6* (whether detected as conversion in tetrads or the production of recombinants among random spores) was accompanied by significantly less than 50% outside marker recombination.

THE *SUP6* locus in *Saccharomyces cerevisiae* was chosen as the subject of a study of intragenic recombination because its extremely high conversion frequency (HURST, FOGEL and MORTIMER 1972) makes it possible to recover all products of conversion from unselected tetrads. It was hoped that, by analyzing all products from a sufficient number of tetrads in which *SUP6* was involved in a recombination event, we could obtain definitive descriptions of parameters such as map expansion and marker effects which have been seen previously only or mainly in selected tetrads or selected intragenic recombinants.

The region of the right arm of linkage group VI which includes *SUP6* is also of interest in that the recombination hot-spot includes several other loci which flank *SUP6*. We hoped to obtain information on the nature of the hot-spot.

The *SUP6* locus codes for an ochre suppressor which substitutes tyrosine in the translation of UAA (GILMORE, STEWART and SHERMAN 1971). The suppressor provides a two-way selective scheme for the detection of mutation and recombination. Mutants of *SUP6* can be selected in a strain carrying a suppres-

sible canavanine resistance, and the reverse selection for recombinants with a functional suppressor makes use of several suppressible auxotrophic mutations.

It was found that, although recombination events affect *SUP6* in over 15% of tetrads, intragenic recombination occurs at a frequency of 10^{-3} or less, so that unselected tetrads cannot be used to describe those events which result in recombination in *SUP6*. For this reason, the tetrad data had to be complemented with results from selected random meiotic products for investigation of some parameters of recombination, and to produce a genetic map of the locus.

MATERIALS AND METHODS

Media: *YEPD*: 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose, 2% Bacto-agar. .1 *YEPD*: yeast extract reduced from 1% to .1%. All other ingredients as in YEPD.

Defined medium: .67% Bacto-yeast nitrogen base without amino acids, 2% dextrose, 2% agar. Adenine, arginine, lysine, histidine, methionine, tryptophan and uracil 20 mg/ leucine 30 mg/ threonine 350 mg in a total of 100 ml stock solution added to a liter of medium. *Omission media*: Defined medium lacking one or more of the above supplements. *-MTh*: double omission medium lacking methionine and threonine, used to score *met10-4*. *-ALT*: triple omission medium lacking adenine, lysine and tryptophan, used to select for functional suppressors. *Canavanine medium*: arginineless omission medium plus 60 or 200 mg filter sterilized canavanine sulfate. *Sporulation media*: Liquid: 1% potassium acetate. Solid: 1% potassium acetate, .25% yeast extract, 2% agar. Supplemented as for defined medium.

Strains: The diploid strains used in this study were all homozygous for the ochre-suppressible alleles *ade2-1*, *lysl-1*, *trp5-48* and *can1-100* and heterozygous for a nonsuppressible allele of *leu2* and *ura4-11*. They carried the following markers as well:

A101:	+	+	*sup6-1-x*	*met10-4*
	his2	*cdc14*	*sup6-1-y*	+
A102:	*his2*	*cdc14*	*sup6-1-x*	+
	+	+	*sup6-1-y*	*met10-4*
A103:	*his2*	*cdc14*	*sup6-1-x*	*met10-4*
	+	+	*sup6-1-y*	+
A104:	+	+	*sup6-1-x*	+
	his2	*cdc14*	*sup6-1*-y	*met10-4*
A75:	+	+	*SUP6-1*	*met10-4*
	his2	*cdc14*	*sup6-1-3*	+
A76:	+	+	*SUP6-1*	*met10-4*
	his2	*cdc14*	*sup6-1-2*	+
A50, A51, A52:	+	+	*SUP6-1*	*met10-4*
	his2	*cdc14*	*sup6+*	+

A811, A011, A021, A022, A031, A032, A041, A042, A161, A181, A182, A183:

+	+	*SUP6-1*	met10-4	*rad*$^-$
his2	*cdc14*	*sup6+*	+	*rad*$^-$

Haploid A14–2B in which secondary mutants of *sup6–1* were selected had the genotype: *SUP6–1, met10–4, ade2–1, lysl–1, trp5–48, can1–100, ura4–11.* The markers and centromere (*) on linkage group VI have the following linkage relationships (MORTIMER and HAWTHORNE 1973):

*	*his2*	*cdc14*	*SUP6*	*met10*
	27	7	4	3

SUP6–1 refers to the ochre suppressor allele at the *SUP6* locus;

sup6–1–x and *sup6–1–y* refer to secondary mutations of *SUP6–1* which no longer have suppressor activity;

sup6+ is the nonsuppressing wild-type allele at that locus.

The sources of the markers used in this study were the following:

X3417–32C: **a**; *asp5–1, trp5–48, arg4–0, lys1–1, his2, cdc14, SUP6–1, met10–4, trp1, ade1* (R. K. MORTIMER);

1403: *α*; *ura4–11* (G. E. MAGNI);

XV162–2C: **a**; *trp5–48, arg4–17, lys1–1, ade2–1, hom3–10* (R. C. VON BORSTEL);

X2280–4A: *α*; *trp5–48, his5–2, lys1–1, ade2–1, ura3–1, met, can1–100, SUP6–1* (R. K. MORTIMER).

One series of diploids was homozygous for one of the following radiation sensitive alleles: *rad1–5, rad2–6, rad3–2, rad4–4, rad16–1, rad18–1.* These alleles came from the strains:

197/2d: *α*; *rad⁻, ade2–1* (B. S. COX);

133/3a: *α*; *rad18–1* (J. C. GAME).

All *rad* alleles except *rad18–1* came from different derivatives of 197/2d.

Selection of secondary mutants of SUP6–1: The haploid strain A14–2B was grown in liquid defined medium lacking adenine, lysine and tryptophan to maintain selection for the suppressor. Then samples were put into tubes of liquid YEPD, allowed to reach stationary phase, and plated on canavanine medium (200 mg/l). Canavanine-resistant colonies were picked, one per tube, and those showing a strict requirement for adenine, lysine and tryptophan were used for further study.

Sporulation and dissection: When meiotic products were to be dissected, the haploid parents were mixed on YEPD and, wherever possible, replica plated to a multiple omission medium selecting for diploids. These were transferred to solid sporulation medium after overnight growth on selective medium and left at 25° for four days or longer before dissection. Asci were digested with a one in twenty dilution of glusulase (Endo) at 37° for 15–30 minutes, then dissected directly on YEPD plates. In later experiments, 0.1 YEPD was used to facilitate development of the red color in adenine-requiring strains.

Tetrads were scored by replica-plating to omission media from the plates on which the tetrads were dissected. If a colony showed color sectoring for the adenine requirement, both halves of the colony were picked and scored separately, to determine whether postmeiotic segregation of *SUP6* had occurred. Postmeiotic segregation for other markers was detected only by the presence of sectoring for growth on an omission medium. It is probable that some sectoring colonies escape detection by this method, so that the numbers for postmeiotic segregation for markers other than *SUP6* represent minimum values only.

Random spore analysis, mating and sporulation: Haploid strains with independently isolated secondary mutations of *SUP6–1* were grown to log phase in liquid YEPD on a shaker at 25°. The two parents were mixed and left shaking for several hours, until they reached the late log or early stationary phase. These mated cultures were washed and resuspended in 1% potassium acetate. They were kept shaking vigorously for four days or more before processing.

Preparation of spore suspension: Sporulated cultures were washed and resuspended in 0.3 ml .5 M sodium thioglycolate in .05 M Tris (pH 8.8) plus 0.05 ml undiluted glusulase. The mixture was incubated for a minimum of one hour at 37°. This suspension was washed and resuspended in 5 ml water. A French Pressure Cell (Aminco, rapid-fill and manual-fill models) was sterilized either by passing steam through the cyclinder for 10 minutes and allowing to cool or by leaving a 1% Roccal solution in the cell for 20–30 minutes, then rinsing thoroughly with sterile distilled water. The suspension was put into the cell, then collected by drops while a pres-

sure of 15,000 PSI was maintained. If by haemocytometer count there remained greater than 1% vegetative cells or groups of spores, the suspension was passed through the pressure cell repeatedly until a count of 99% single spores was achieved.

Selection of meiotic recombinants: The samples prepared by the technique above were plated on complete defined medium and on –ALT triple omission medium to select for strains with a functional suppressor. Plates were incubated at 25° for seven days before counting. Recombinants were picked and tested for the markers flanking *SUP6*, that is, histidine and methionine requirements and temperature sensitivity (growth on YEPD at 36°).

Postmeiotic segregation of *SUP6* was identified as sectoring of spore colonies for the red color characteristic of *ade2* strains. The classification was confirmed if, on cloning and retesting, the colonies contained a mixture of cells with and without *SUP6*, but were not mixed for the unlinked markers *ura4* and *leu2*.

RESULTS

Conversion and co-conversion as determined by tetrad analysis: The dissection of 2627 complete unselected tetrads heterozygous for *SUP6* gave the classes of products listed in Table 1. Figure 1 summarizes the frequencies of single site

TABLE 1

Genotypes: $\frac{his2 \quad cdc14 \quad + \quad +}{+ \;{}_1\; + \;{}_2\; SUP6\text{–}1 \;{}_3\; met10}$

(Diploids A50, A51, A52, A53, A811, A021, A022, A031, A032, A041, A042, A161, A181, A182, and A183)

or $\frac{his2 \quad cdc14 \quad sup6\text{–}1\text{–}x \quad +}{+ \;{}_1\; + \;{}_2\; SUP6\text{–}1 \;{}_3\; met10}$

(Diploid A76 carries *sup6–1–2* and A75 carries *sup6–1–3*)

	Direction of conversion of *SUP6**	Region of reciprocal exchange	Event at *SUP6* involved in crossover?†	*SUP6–1* / +	*sup6–1–2* / *SUP6–1*	*sup6–1–3* / *SUP6–1*	Total
NO CONVERSION							
1. ++—— ——++ ++—— ——++				1209	183	173	1565
Single exchange							
2. ++—— ——++ —+—— +—++		1		86	12	13	111
3. ++—— ——++ ———— ++++		2		16	5	2	23
4. ++—— ——++ ——+— ++—+		3		39	12	11	62
			TOTAL	141	29	26	196
Multiple exchange							
5. —+—— +—++ —+—— +—++		1,1		1	0	0	1
6. ——++ —+—— +——— ++++		1,2		2	0	0	2
7. ++—— ——++ —+++ +———		1,2		0	1	1	2
8. ++—— —+++ +—++ ————		1,2		1	0	0	1
9. ++—— ——++ ———+ +++—		2,3		0	0	1	1
10. ——++ ++—+ +++— ————		2,3		0	0	1	1
11. ——++ —+—— +—+— ++—+		1,3		1	0	0	1
12. ++—— ——++ +—+— —+—+		1,3		1	0	0	1
			TOTAL	6	1	3	10
CONVERSION OF *SUP6* ALONE							
13. ++—— ——++ ——++ +++—	+			43	10	4	57
14. ++—— ——++ ++—— ———+	—			49	11	10	70
15. ++—— ——++ —++— +—++	+	1		1	0	1	2
16. ——++ —+—— +—++ +++—	+	1		1	0	0	1
17. ++—— ——++ —+—— +——+	—	1		5	1	0	6
18. ++—— —+—— +—++ ———+	—	1		2	0	0	2

TABLE 1—Continued

		Direction of conversion of *SUP6**	Region of reciprocal exchange	Event at *SUP6* involved in crossover?†	*SUP6–1* +	*sup6–1–2* *SUP6–1*	*sup6–1–3* *SUP6–1*	Total
19.	++—— ——++ ——+— ++++	+	2 or 3	yes	23	2	8	33
20.	++—— ——++ ———— ++—+	—	2 or 3	yes	21	6	8	35
21.	——++ ——+— ++—+ +++—	+	3	no	1	0	0	1
22.	++—— ———+ ——+— ++—+	—	3	no	1	0	0	1
23.	++—— +—++ —+++ ——+—	+	1,2 or 1,3	yes	1	0	0	1
24.	——++ —+—— +—+— ++++	+	1,2 or 1,3	yes	1	0	0	1
25.	++—— ——++ —+++ +—+—	+	1,2 or 1,3	yes	2	0	0	2
26.	——++ —++— +———— ++++	+	1,2	no	0	1	0	1
27.	++—— +——+ —+—+ ——+—	—	1,3	no	1	0	0	1
28.	——+— ——+— ++—+ ++++	+	2,3 or 3,3	yes	1	0	0	1
29.	++—+ ++—+ ——+— ————	—	2,3 or 3,3	yes	1	0	0	1
				TOTAL	154	31	31	216
SUP6 4:0 OR 0:4								
30.	——++ ——++ +++— +++—	+,+			0	1	0	1
31.	++—— ++—— ———+ ———+	—,—			0	0	1	1
32.	——++ ——+— +++— ++++	+,+	2 or 3	yes,no	1	1	0	2
33.	++—— ———+ ++—+ ————	—,—	2 or 3	yes,no	2	0	1	3
				TOTAL	3	2	2	7
POSTMEIOTIC SEGREGATION OF *SUP6*								
34.	++—— ——++ ——++ ++P—				3	0	1	4
35.	++—— ——++ ++—— ——P+				3	0	1	4
36.	++—— ——++ ++—+ ——P—		3	yes	1	3	1	5
37.	++—— ——++ ——+— ++P+		3	yes	1	0	0	1
38.	++—— ——++ ++++ ——P—		2	yes	0	0	2	2
39.	++—— ——++ —+—— +—P+		1		1	0	0	1
40.	——++ ———+ ++++ ++P—‡		2,3	no	1	0	0	1
41.	——++ ———+ +—+— ++P—‡		2,3	no	1	0	0	1
42.	++—— ——++ —+++ +—P—		1,2	yes	0	0	1	1
43.	++—— ——++ ——++ ++PP				1	0	0	1
44.	++—— ——++ ++—— P—P+				0	1	0	1
45.	++—— ——++ +—++ —PP—		1	yes	1	0	0	1
46.	++—— ——++ ++—— PPP+				2	0	0	2
47.	++—— —+—— +—++ PPP+		1	no	1	0	0	1
48.	++—+ ++—+ ——P— ——P—		2,3 or 2,2 or 3,3	both	1	0	0	1
49.	——+— ———— ++P+ ++P+		2,3	both	1	0	0	1
50.	++—— ——++ +PP+ —+PP		1	yes,no	1	0	0	1
51.	++—— ——++ ++—— —+P+§				2	0	1	3
52.	++—— ——++ ++—— ——P—§				0	0	1	1
53.	++—— ——++ ——++ ++P+§				2	0	0	2
54.	++—— ——++ ——++ +—P+§				1	0	0	1
55.	++—— ——++ ++—— —+P—§				1	0	0	1
56.	++—— ——++ ++++ ——P+§		2	yes	1	0	0	1
57.	++—— ——++ —+—— ++P+§		1 or 2	yes	3	0	0	3
58.	++—— ——++ ++—+ —+P—§		3	yes	1	0	1	2
59.	——++ ——++ +++— ++P—§,¶	+			2	0	0	2
60.	——++ ——++ +—++ —PP—‖	+			0	1	0	1
61.	++—— ——++ ———+ ——P—§,¶	—			0	1	0	1
62.	++—— ——++ ——P— ——P+§		2 or 3	yes	1	0	0	1
63.	++—— ——++ ++—— +—P+‡				1	0	0	1
64.	++—— ——++ +—++ ——PP‡		2	yes	1	0	0	1
65.	——++ —++— ++++ ——P—‡,¶		2	yes,no	0	0	1	1
66.	++—— ——+— +—++ ++P—‡		1		0	0	1	1
				TOTAL	35	6	11	52
CONVERSION OF *SUP6* WITH INDEPENDENT CONVERSION OF *his2*								
67.	——++ ——++ —+—— +++—	+			3	3	0	6
68.	++—— ——++ +—++ +++—	+			3	2	0	5
69.	++—— ——++ ——++ —++—	+			2	0	0	2
70.	——++ ——++ —++— +++—	+,+			1	0	0	1

TABLE 1—Continued

	Direction of conversion of *SUP6**	Region of reciprocal exchange	Event at *SUP6* involved in crossover?†	*SUP6–1* +	*sup6–1–2* *SUP6–1*	*sup6–1–3* *SUP6–1*	Total
71. ++—— ——++ ——+ —+——	—			3	0	0	3
72. ++—— ++—— +—++ ——+	—			1	0	1	2
73. ++—— ——++ ++—— +——+	—			1	0	0	1
74. ++—— +—++ +—++ —++—	+	1		0	1	0	1
75. ++—— +—++ ——+— ++++	+	2 or 3	yes	1	0	0	1
76. ++—— ——++ +—+— ++++	+	2 or 3	yes	1	0	0	1
77. ++—— ——++ +—— ++—+	—	2 or 3	yes	1	0	0	1
78. ++—— ——— +—++ ++—+	—	2 or 3	yes	1	0	0	1
79. ——++ ++—+ —+—— ———	—	2 or 3	yes	0	0	1	1
80. ++—— ——++ —+—+ ———	—	2 or 3	yes	0	0	1	1
81. ——++ —+—— ——+— ++++	+	2 or 3	yes	3	0	0	3
82. ++—— ——++ ——+ ———**	—			0	1	0	1
			TOTAL	21	7	3	31
CONVERSION OF *SUP6* WITH INDEPENDENT CONVERSION OF *cdc14*							
83. ++—— ——++ —+++ +++—	+			0	1	0	1
84. ++—— ++—— —+++ —+—+	—			0	0	1	1
85. ++—— ——++ ——— +——+	+	2 or 3	yes	1	0	0	1
86. ++—— ——++ —++— ++++	+	2 or 3	yes	1	0	1	2
87. ++—— ——+— —+++ ++++	+	2 or 3	yes	1	0	0	1
88. ++—— ——+ +—++ ———	—	2	no	1	0	0	1
			TOTAL	4	1	2	7
CONVERSION OF *SUP6* WITH INDEPENDENT CONVERSION OF *met10*							
89. ++—— ——++ ——+— +++—	+			3	0	0	3
90. ——++ ——++ ++—+ +++—	+			0	0	1	1
91. ++—— ++—— ——+ ——+—	—			1	0	0	1
92. ++—— ——++ ——+ ++—+	—			2	0	1	3
93. ++—— ——+— ——+— ++++	+	2 or 3	yes	0	0	1	1
94. ++—— ——++ ++++ ——+P	+	2 or 3	yes	1	0	0	1
95. ++—P ——++ ++—+ ———	—	2 or 3	yes	1	0	0	1
			TOTAL	8	0	3	11
SUP6 4:0 OR 0:4 WITH CONVERSION OF AN ADJACENT LOCUS							
96. ——++ ——++ +++— ++++	+			0	1	0	1
97. ——++ ——++ +—+— +++—	+			1	0	0	1
98. ——++ ——++ ——+— +++—	+			1	0	0	1
99. ++++ ++++ ++++ ++++	+			1	0	0	1
100. ++—— ++—— —+—+ ——+	—			1	0	0	1
101. ——++ +—++ +++— ——+—	+	3	yes,no	0	1	0	1
102. ++—— —+—— —+—+ +——+	—	1		1	0	0	1
103. ++—— ——— +——+ ++—+	—	2 or 3	yes,no	1	0	0	1
104. ++—— ++—+ —+—— ——+	—	3	yes,no	1	0	0	1
			TOTAL	7	2	0	9
CO-CONVERSION OF *SUP6* AND *cdc14*							
105. ++—— ——++ ——++ +—+—	+			8	0	3	11
106. ++—— ——++ ++—— —+—+	—			6	2	3	11
107. ++—— ++—— —+—+ ——+—‡	—			0	0	1	1
108. ++—— ——++ —+—— —+—+‡	—			0	1	0	1
109. ++—— ——++ —+—+ ++—+‡	—			1	0	1	2
110. ++—— ——+— —+—+ ++—+	—	3	no	0	0	1	1
111. ++—— ——++ ——+— +—++	+	3	yes	10	0	2	12
112. +—++ —+—— ——+— +—++	+	1,3	yes	0	1	0	1
113. ++—— ——++ ++—+ —+——	—	3	yes	11	4	7	22
114. ++—— —+—— ——+ ++++	—	2,3	no,yes	1	0	0	1
			TOTAL	37	8	18	63
CO-CONVERSION OF *SUP6* AND *met10*							
115. ++—— ——++ —+++ ++++‡	+			0	0	1	1
116. ++—— ——++ ——++ ++++	+			4	2	2	8

TABLE 1—Continued

	Direction of conversion of *SUP6**	Region of reciprocal exchange	Event at *SUP6* involved in crossover?†	*SUP6-1* +	*sup6-1-2* *SUP6-1*	*sup6-1-3* *SUP6-1*	Total
117. ++—— ——++ ——++ +++P	+			1	0	0	1
118. ——++ ——++ —+—— ++++‡	+			1	0	0	1
119. ++—— ——++ ++—— ————	—			2	1	3	6
			TOTAL	8	3	6	17
CO-CONVERSION OF *SUP6*, *his2* AND *cdc14*							
120. ++—— ——++ ——++ ——+—	+			2	1	1	4
121. ++—— ——++ ——+— ——+—‡	+			0	0	1	1
122. ++—— ——++ ++—— ++—+	—			3	1	0	4
123. ——++ ——++ ——+— +++—¶	+			1	0	0	1
124. ++—— ——++ ++—+ ++—+‡	—			1	0	0	1
125. ——++ ——+— ——+— ++—+	+	3	no	1	0	0	1
126. ++—— ++—+ +—++ —+——	—	1		2	0	0	2
127. ++—— ——+— +—++ PP++††	+	2 or 3	yes	0	1	0	1
			TOTAL	10	3	2	15
CO-CONVERSION OF *SUP6*, *cdc14* AND *met10*							
128. ++—— ——++ ——++ +—++	+			7	1	0	8
129. ++—— ——++ +—++ +—++‡	+			1	0	1	2
130. ——++ ——++ —+—— +—++‡	+			1	0	0	1
131. ++—— ——++ ++—— —+——	—			1	2	1	4
132. ++—— ++—— —+—— +—++‡	—			0	0	1	1
133. ++—— —+—— —+—— +—++	—	1	no	1	0	0	1
			TOTAL	11	3	3	17
CO-CONVERSION OF *SUP6*, *his2*, *cdc14* AND *met10*							
134. ++—— ——++ ——++ ——++	+			0	3	0	3
135. ++—— ——++ ++—— ++——	—			3	1	3	7
136. ——++ ——++ ——++ ——++	+,+			1	0	0	1
137. ++—— ++—— ++—— ++——	—,—			1	0	0	1
			TOTAL	5	4	3	12
CONVERSION OF *his2*							
138. ++—— ——++ ++—— +—++				85	9	10	104
139. ++—— ——++ ——++ —+——				86	19	16	121
140. ++—— ——++ +—+— ++——‡				1	0	0	1
141. ++—— +—++ +—++ —+——		1		5	0	0	5
142. ——++ —+—— —+—— +—++		1		2	0	0	2
143. ++—— ——++ —+++ ————		2		1	0	0	1
144. ——++ ——+— —+—— ++—+		3		4	1	0	5
145. ++—— ——++ —+—+ ——+—		3		0	0	1	1
146. ++—— ——+— +——+ ++++		2,3		1	0	0	1
			TOTAL	185	29	27	241
his2 4:0 OR 0:4							
147. ++—— ++—— +—++ +—++				3	0	2	5
148. ——++ ——++ —+—— —+——				2	1	0	3
149. ——++ ——+— —+—— —+—+		3		1	0	0	1
			TOTAL	6	1	2	9
CONVERSION OR POSTMEIOTIC SEGREGATION OF *cdc14*							
150. ++—— ——++ ++—— —+++				9	0	7	16
151. ++—— ——++ ——++ +———				9	0	2	11
152. ——++ —+—— +——— +—++		1		1	0	0	1
153. ++—— ——++ —+—— ++++		1 or 2		9	0	2	11
154. ++—— ——++ ———— +—++		1 or 2		10	0	1	11
155. ++—— ——+— —+++ ++—+		3		1	0	0	1
156. +—++ —+—— —+—— ++++		1,1 or 1,2		1	0	0	1
157. ++—— ——+— —+—+ ++++		1,3 or 2,3		2	0	0	2
158. ——++ +++— ———+ +———		2,3		1	0	0	1
159. ++—— ——++ ++—— —P++				1	0	0	1
160. ++—— ——++ +—++ —P——		1		1	0	0	1
			TOTAL	45	0	12	57

TABLE 1—Continued

	Direction of conversion of *SUP6**	Region of reciprocal exchange	Event at *SUP6* involved in crossover?†	*SUP6-1* / +	*sup6-1-2* / *SUP6-1*	*sup6-1-3* / *SUP6-1*	Total
CO-CONVERSION OF *his2* AND *coc14*							
161. ++—— ——++ ++—— ++++				2	3	0	5
162. ++—— ——++ ——++ ————				1	2	1	4
163. ——++ —+—— +—++ ————		1		1	0	0	1
			TOTAL	4	5	1	10
CONVERSION OF *met10*							
164. ++—— ——++ ——++ ++—+				19	6	5	30
165. ++—— ——++ ++—— ——+—				15	3	3	21
166. ++—— ——++ —+—+ +—++		1		1	0	0	1
167. ——++ —+—— +—++ ++—+		1		2	1	0	3
168. ++—— ——++ —+—— +—+—		1		1	0	0	1
169. ++—— ——+— —+—— +—++		1		0	0	1	1
170. ++—— ——++ ———+ ++++		2		1	0	0	1
171. ++—— ——++ ———— +++—		2		1	0	0	1
172. ++—+ ——++ ++—+ ——+—		3		2	1	0	3
173. +—++ +—++ —+—+ —+——		1,1		1	0	0	1
			TOTAL	43	11	9	63
INDEPENDENT CONVERSIONS OF TWO MARKERS NOT AFFECTING *SUP6*							
174. ++—— ——++ ——++ —+—+				1	0	0	1
175. ++—— ——+— ——+— —+——				1	0	0	1
176. ++—— ——++ ——+— —+——				1	0	0	1
177. ——++ ——++ —+—— ++—+				1	0	0	1
178. ++—— ——++ +—++ ++—+				0	1	1	2
179. ——++ —+—+ ——+— ++—+		3		1	0	0	1
180. ++—— ——++ —+—— —+++				2	0	0	2
181. ++—— ——++ +——— +—++				1	1	0	2
182. ——++ ——++ —+—— +———				1	0	0	1
183. ++—— ——++ ——+— +———				0	0	1	1
184. ++—— ——++ —+++ ++—+				1	0	0	1
185. ——++ +—++ ++—+ ————		2		0	1	0	1
186. ++—— ——++ —+—+ ++++		2		0	1	0	1
187. ++—— ———+ —++— +++—		2,3		0	0	1	1
			TOTAL	10	4	3	17
INDEPENDENT CONVERSIONS OF THREE MARKERS							
188. ++—— ——++ +——+ ———+	—			0	0	1	1
189. ——++ ——++ —+—— +——+				1	0	0	1
			TOTAL	1	0	1	2
			TOTAL OF ALL CLASSES	1953	333	341	2627

* For *SUP6*, + refers to the wild-type phenotype, without suppression;
— refers to the suppressor phenotype.

† "yes" means the chromatid which showed conversion or postmeiotic segregation for *SUP6* was involved in the crossover; "no" means it was not; ascus types with two chromatids involved in the *SUP6* event are marked "yes,no" or "no,yes" or "both" (meaning yes,yes).

‡ Tetrads include a conversion independent of the event classified.

§ Tetrads with conversion linked to the postmeiotic segregation at *SUP6*.

¶ Tetrads with conversion of *SUP6* in addition to the event classified.

‖ This tetrad has co-conversion involving *SUP6* and conversion linked to co-postmeiotic segregation at *SUP6* and *cdc14*.

** Tetrad includes independent co-conversion.

†† This tetrad was interpreted as co-postmeiotic segregation linked to a conversion at *SUP6* and a crossover in region 3, with independent conversion to + of *his2*.

conversions and the frequency of tetrads with co-conversion of adjacent marked loci excluding 4:0 and 0:4 segregations. An event was classified as a co-conversion only if adjacent markers were converted on the same chromatid in the same direction.

The number of tetrads of this pattern which would be expected to arise by coincident but independent conversions of the two markers would be low. There were 42 detectable independent conversions of adjacent markers. This number does not differ significantly from that expected from the products of conversion frequencies for single markers.

Table 2 summarizes the numbers and types of aberrant segregations for all markers. The frequency of conversion from mutant to wildtype and wildtype to mutant was equal for all alleles tested except *met10–4*. There was also an overall equality of post-meiotic segregation of chromatids carrying mutant alleles and wild-type alleles, as seen in the 3:5 and 5:3 segregations, although the number for any one allele except *SUP6–1* was too small to make a statistical comparison. For *SUP6–1*, there were 15 postmeiotic segregations of *SUP6–1* carrying chromatids and 22 postmeiotic segregations of *sup6*+ chromatids.

False tetrads: In cross A75, 106 tetrads with conversion of one of the regularly scored markers were tested for segregation of mating type. Scoring was done microscopically by looking for zygotes in a mixture of the colony being tested and a tester strain of each mating type. Of 100 tetrads in which all four spores were scored, all showed 2:2 segregation for mating type. Six tetrads were incompletely scored because of poor mating of one or more spores. On the least favorable assumption that false clusters involve three spores from one tetrad and one unrelated spore, 50% of false clusters are expected to show aberrant segregation for any one marker. Since this study showed no aberrant segregation for mating

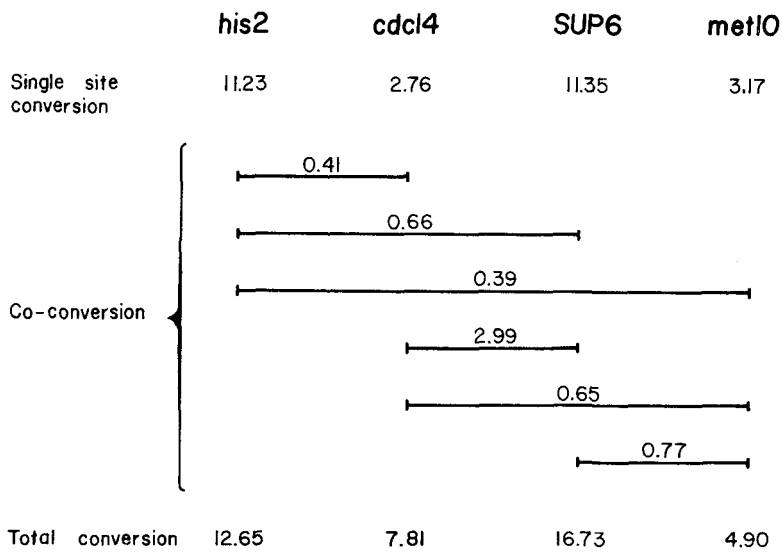

FIGURE 1.—Percent tetrads with single and multiple site conversions.

TABLE 2

Number of tetrads with aberrant segregation for all markers tested‡

Ratio *wild-type*: mutant	3:1	1:3	4:0	0:4	5:3	3:5	2:6[ab]	4:4[ab]	7:1	1:7	Number of tetrads	Percent tetrads with aberrant segregation
his2	182	205	7	8	5	0	0	0	0	0	3115	13.07
cdc14	132	107	3	1	5	2	0	0	0	1	3167	7.93
SUP6–1	128	126	7	7	12	17	1*	3†	2	0	1952	15.52
sup6–1–2	29	31	4	0	3	2	0	0	1	0	333	21.02
sup6–1–3	27	43	0	2	5	5	0	0	1	0	341	24.34
met10–4	92	53	1	2	5	0	0	0	0	0	3063	5.00
ura4–11	23	17	28	22	0	1	0	0	0	0	3409	2.67
leu2	98	96	1	2	1	1	0	0	0	0	3407	5.72

* *SUP6–1/sup6+*
SUP6–1/SUP6–1
SUP6–1/sup6+
SUP6–1/SUP6–1
† *SUP6–1/SUP6–1*
SUP6–1/sup6+
SUP6–1/sup6+
sup6+/sup6+

‡ The techniques used would not reliably detect that a colony was of mixed genotype for any markers except those at *SUP6*. The numbers given are those which were detected.

type in 100 tetrads, it may be stated with 95% confidence that there were fewer than 6% false tetrads among asci which showed conversion for *his2*, *cdc14*, *SUP6*, *met10*, *ura4* or *leu2*. The genotypes of the unscored spores made it highly unlikely that they were diploid; they all showed 2:2 segregation for at least three other markers.

Outside marker recombination: Tables 3, 4 and 5 show the number of conversions of *SUP6* alone, co-conversions of *cdc14* and *SUP6*, and conversions of *cdc14* alone, classified as to whether these were or were not accompanied by outside marker exchange involving the converted chromatid. Conversion of *SUP6* alone showed 37% associated recombination of outside markers. The difference from 50% is highly significant ($\chi^2=20.2$; $p \ll 0.001$). For that same set of tetrads, conversion of *cdc14* was accompanied by 50% flanking marker recombination, and the longer co-conversion events involving both loci had more recombinant products than parental ($\chi^2_1=8.6$; $p=0.005–0.001$). Total conversions of *SUP6*, including conversions of *SUP6* alone, and co-conversions with *cdc14*, still showed a significant excess of noncrossover tetrads, 205 *SUP6* convertants with parental flanking markers and 154 with the recombinant class, 43% outside marker exchange ($\chi^2_1=7.2$; $p=0.01–0.005$). These patterns are independent of sporulation frequency which ranged from approximately 27% (7% three and four spored asci) in cross A161, to 61% (36% three and four spored asci) in A51.

TABLE 3

Configuration of outside markers in spores with conversion or postmeiotic segregation of SUP6

Genotype	Diploid	Number of tetrads	Parental			Recombinant			Not determined	Omitted
			Number of conversions with parental outside markers	Unrelated crossover between *cdc14* and *met10*	Unrelated crossover between *his2* and *cdc14*	Number of conversions with recombinant outside markers	Unrelated crossover between *cdc14* and *met10*	Unrelated crossover between *his2* and *cdc14*	Conversion of flanking marker	0:4 or 4:0 segregation for *SUP6*
SUP6-1 × *sup6+*										
1. *RAD+*	A50	149	6	.	.	4	.	.	3	.
RAD+	A51	148	11		1	4			2	
RAD+	A52	130	10			2			4	
RAD+	A53	19	2			1			0	
2. *rad1-18*	A811	178	9		1	8	1	1	0	1
rad1-5	A011	166	10			4			0	
rad2-6	A021	172	8			2	1	1	1	
	A022	110	10		1	3			1	
rad3-2	A031	104	7	1	1	4	1	1	2	
	A032	145	8		1	8	1		1	1
rad4-4	A041	140	10	1	1	6			1	1
	A042	52	4			2			1	
rad16-1	A161	157	10		2	2			1	1
rad18-1	A181	60	5	1	1	3			2	
	A182	90	9		1	10		1	0	
	A183	132	10	1		6		1	1	1
sup6-1-x × *SUP6-1*										
3. *sup6-1-3*	A75	341	22	1		25		1	9	2
sup6-1-2	A76	333	30	1	3	11		1	3	3
			181	6	13	105	4	7	32	10

TABLE 4

Configuration of outside markers in spores with co-conversion of SUP6 *and* cdc14

Genotype	Diploid	Number of tetrads	Parental: Number of conversions with parental outside markers	Parental: Unrelated crossover between *his2* and *met10*	Recombinant: Number of conversions with recombinant outside markers	Recombinant: Unrelated crossover between *his2* and *met10*	Not determined: Conversion of flanking marker	Omitted: 0:4 or 4:0 segregation for *SUP6* and/or *cdc14*
SUP6-1 × *sup6+*								
1. *RAD+*	A50	149	3		5		1	
RAD+	A51	148	0		2		3	
RAD+	A52	130	1		2		2	
RAD+	A53	19	0		0		0	
2. *rad1-18*	A811	178	2		3		2	1
rad1-5	A011	166	1		0		1	
rad2-6	A021	172	1		0		2	2
	A022	110	1		2		1	1
rad3-2	A031	104	0		1		2	
	A032	145	1		1		2	
rad4-4	A041	140	0		6		3	1
	A042	52	2		1		3	
rad16-1	A161	157	0		0		2	
rad18-1	A181	60	0		1		0	
	A182	90	1		1		1	1
	A183	132	3		5		1	2
sup6-1-x × *SUP6-1*								
3. *Sup6-1-3*	A75	341	6		10		9	1
Sup6-1-2	A76	333	2		9	1	12	1
			24	0	49	1	47	10

Only those events which did not involve co-conversion of a flanking marker have been tabulated here, since this makes it impossible to tell whether or not the event was associated with a crossover.

The observation of less than 50% outside marker recombination among *SUP6* convertants is confirmed in random spore analysis where the observation is extended to include all combinations of alleles (Table 6). In heteroallelic crosses in which selection for a functional suppressor required a recombination event within the *SUP6* locus, the percent recombination of flanking markers ranged from 32% to 47% with an average of 39%. In all 48 heteroallelic crosses, the fraction of recombinant outside marker configurations was smaller than the fraction of parental outside marker configurations, and in 44 of these, the difference between the two classes was significant at the 5% level as determined by a goodness-of-fit chi square test (Table 6, column 4).

TABLE 5

Configuration of outside markers in spores with conversion of cdc14

Genotype	Diploid	Number of tetrads	Parental		Recombinant		Not determined	Omitted
			Number of conversions with parental outside markers	Unrelated crossover between *his2* and *SUP6*	Number of conversions with recombinant outside markers	Unrelated crossover between *his2* and *SUP6*	Conversion of flanking marker	0:4 or 4:0 segregation for *cdc14*
SUP6–1 × *sup6+*								
1. *RAD+*	A50	149	2		1		1	
RAD+	A51	148	0		3		0	
RAD+	A52	130	5		1		2	
RAD+	A53	19	0		0		1	
2. *rad1–18*	A811	178	1		3		1	
rad1–5	A011	166	2	1	1		0	
rad2–6	A021	172	1	1	0		1	
	A022	110	1		0		0	
rad3–2	A031	104	2		3		1	
	A032	145	1		2		1	
rad4–4	A041	140	0		2		2	
	A042	52	2		1		0	
rad16–1	A161	157	2		1		0	
rad18–1	A181	60	1		2		1	
	A182	90	0		3		0	
	A183	132	3		3	1	1	
sup6–1–x × *SUP6–1*								
3. *sup6–1–3*	A75	341	12		3		1	
sup6–1–2	A76	333	0		2		6	
			35	2	31	1	19	0

The relative frequencies of recombinant *versus* parental classes of outside markers appear to be characteristics of an allele pair. Heterogeneity in the proportion of parental and recombinant classes was less among repeated crosses with the same alleles and different arrangements of outside markers than the heterogeneity among crosses with other allelic combinations.

Mapping from the inequality of recombinant outside marker combinations among selected prototrophs: The results of random spore analysis of all pairwise crosses of strains carrying one of 10 secondary mutations of *SUP6–1* or the wild-type allele at that locus are presented in Table 7. The designation AC is given to the wild-type allele at the site of the original *SUP6–1* mutation (HAWTHORNE and LEUPOLD 1974). This table shows the prototroph frequencies and the distribution of outside markers among selected prototrophic random meiotic prod-

TABLE 6

Fraction P /P, R /R and R/Total for crosses involving alleles which map at different sites

	P_1/P_1+P_2	R_1/R_1+R_2	Recombinant/ total	Number analyzed
1–7 × 1–2	.529	.624	.435	526
1–7 × 1–6	.500	.611	.427	1169
1–7 × 1–8	.559	.732	.415	306
1–7 × 1–10	.526	.695	.329	259
1–7 × 1–11	.541	.662	.365	381
1–7 × 1–5	.415*	.733	.348	742
1–7 × AC	.615*	.820	.389	599
1–7 × 1–3	.511	.735	.467†	492
1–7 × 1–9	.586*	.776	.406	594
1–7 × 1–20	.497	.796	.460†	298
1–2 × 1–5	.466	.719	.368	533
1–2 × AC	.586*	.731	.376	593
1–2 × 1–3	.435*	.536†	.422	429
1–2 × 1–9	.439*	.665	.355	597
1–2 × 1–20	.381*	.793	.387	287
1–6 × 1–8	.510	.560†	.342	292
1–6 × 1–10	.489	.647	.394	216
1–6 × 1–11	.536	.658	.378	294
1–6 × 1–5	.517	.740	.351	593
1–6 × AC	.601*	.754	.382	552
1–6 × 1–3	.468	.725	.405	582
1–6 × 1–9	.567	.783	.392	293
1–6 × 1–20	.544	.760	.422	592
1–8 × 1–5	.446	.556†	.316	285
1–8 × AC	.538	.712	.418	457
1–8 × 1–3	.424	.654	.361	144
1–8 × 1–9	.491	.726	.420	295
1–8 × 1–20	.427*	.770	.379	792
1–10 × 1–5	.375*	.659	.382	961
1–10 × AC	.486	.716	.389	525
1–10 × 1–3	.469	.640	.394	289
1–10 × 1–9	.686*	.762	.423	298
1–10 × 1–20	.436	.847	.371	299
1–11 × 1–5	.358	.616	.330	221
1–11 × AC	.539	.735	.406	577
1–11 × 1–3	.415*	.584†	.342	260
1–11 × 1–9	.428*	.632	.345	496
1–11 × 1–20	.424*	.841	.359	298
1–5 × AC	.547	.672	.370	470
1–5 × 1–3	.378*	.519†	.393	466
1–5 × 1–9	.497	.763	.389	293
1–5 × 1–20	.433	.780	.340	294
AC × 1–3	.279*	.515†	.391	253
AC × 1–9	.496	.818	.385	200
AC × 1–20	.528	.796	.386	293
1–3 × 1–9	.705*	.696	.389	144
1–3 × 1–20	.654*	.720	.386	259
1–9 × 1–20	.512	.742	.446†	296
Mean	.496	.703	.386	

* Significantly different from 50% at 5% level.
† Not significantly different from 50% at 5% level.

TABLE 7

Meiotic recombination frequencies and distribution of outside markers among recombinants

Alleles	Prototroph frequency $\times 10^6$	Number of repeats	Range of values	P_1	P_2	R_1	R_2
1–7 × *1–7*	4	1		35	26	5	4
1–7 × *1–2*	1227	2	1150–1350	157	140	143	86
1–7 × *1–6*	1237	5	867–1715	335	335	305	194
1–7 × *1–8*	614	1		100	79	93	34
1–7 × *1–10*	800	1		102	92	66	29
1–7 × *1–11*	568	1		131	111	92	47
1–7 × *1–5*	171	4	115–305	201	283	189	69
1–7 × *AC*	864	5	631–1281	225	141	191	42
1–7 × *1–3*	861	2	852–880	134	128	169	61
1–7 × *1–9*	1156	2	1034–1654	207	146	187	54
1–7 × *1–20*	2384	1		80	81	109	28
1–2 × *1–2*	12	1		90	102	33	43
1–2 × *1–6*	80	2	54–89	165	154	90	63
1–2 × *1–8*	9	2	7–11	15	20	4	4
1–2 × *1–10*	26	1		56	88	64	30
1–2 × *1–11*	26	2	26–27	82	102	77	22
1–2 × *1–5*	307	2	241–368	157	180	141	55
1–2 × *AC*	232	6	180–320	217	153	163	60
1–2 × *1–3*	208	2	175–239	108	140	97	84
1–2 × *1–9*	694	2	497–793	169	216	141	71
1–2 × *1–20*	416	1		67	109	88	23
1–6 × *1–6*	14	1		45	71	19	17
1–6 × *1–8*	136	1		98	94	56	44
1–6 × *1–10*	236	1		64	67	55	30
1–6 × *1–11*	225	1		98	85	73	38
1–6 × *1–5*	467	2	380–501	199	186	154	54
1–6 × *AC*	985	2	865–1386	205	136	159	52
1–6 × *1–3*	418	2	369–456	162	184	171	65
1–6 × *1–9*	348	1		101	77	90	25
1–6 × *1–20*	1207	2	1059–1858	186	156	190	60
1–8 × *1–8*	18	1		8	12	2	3
1–8 × *1–10*	16	2	15–48	57	44	28	15
1–8 × *1–11*	13	1		7	6	6	0
1–8 × *1–5*	240	1		87	108	50	40
1–8 × *AC*	241	4	193–298	143	123	136	55
1–8 × *1–3*	29	4	19–80	39	53	34	18
1–8 × *1–9*	281	1		84	87	90	34
1–8 × *1–20*	279	3	168–696	210	282	231	69
1–10 × *1–10*	15	2	13–29	18	12	3	2
1–10 × *1–11*	72	2	40–101	48	51	15	13
1–10 × *1–5*	189	6	155–459	223	371	242	125
1–10 × *1–AC*	149	3	140–169	156	165	146	58
1–10 × *1–3*	158	1		82	93	73	41
1–10 × *1–9*	392	1		70	102	96	30
1–10 × *1–20*	733	2	731–736	82	106	94	17
1–11 × *1–11*	2	2	1–11	7	10	2	2
1–11 × *1–5*	200	1		53	95	45	28
1–11 × *AC*	161	3	114–233	185	158	172	62

TABLE 7—Continued

Alleles	Prototroph frequency $\times 10^6$	Number of repeats	Range of values	P_1	P_2	R_1	R_2
1–11 × *1–3*	208	1		71	100	52	37
1–11 × *1–9*	376	3	167–716	139	186	108	63
1–11 × *1–20*	630	1		81	110	90	17
1–5 × *1–5*	11	1		17	23	4	14
1–5 × *AC*	138	3	88–182	162	134	117	57
1–5 × *1–3*	173	2	118–343	107	176	95	88
1–5 × *1–9*	256	1		89	90	87	27
1–5 × *1–20*	528	1		84	110	78	22
AC × *AC*	1	2	1–3	7	15	3	0
AC × *1–3*	285	4	238–692	43	111	51	48
AC × *1–9*	7	4	4–18	61	62	63	14
AC × *1–20*	308	1		95	85	90	23
1–3 × *1–3*	67	1		102	113	26	40
1–3 × *1–9*	618	1		62	26	39	17
1–3 × *1–20*	871	1		104	55	72	28
1–9 × *1–9*	0	1		3	5	1	0
1–9 × *1–20*	105	1		84	80	98	34
1–20 × *1–20*	3	1		32	28	7	10

ucts. For crosses which were repeated, the prototroph frequencies are weighted averages.

Given the diploid $\frac{\text{A}\quad 1+\quad \text{B}}{\text{a}\quad +2\quad \text{b}}$

R_1 is the more common recombinant class among selected intragenic recombinants, expected from a single crossover between the alleles or a conversion with an adjacent crossover. a ++ B.

R_2 is the rare recombinant class. Seen as a triple exchange, it requires conversion of one allele with crossingover removed from the site of conversion. A ++ b.

P_1 is the parental combination of outside markers which entered the cross with the proximal allele. A ++ B.

P_2 is the parental combination of outside markers which entered the cross with the distal allele. a ++ b.

The proximal or distal position of an allele and thus P_1 and P_2 are determined from the order suggested by the R_1:R_2 inequality. The allele order is that which makes the more common recombinant class the "single-exchange" class.

Two features of the results should be pointed out before discussing the characteristics of meiotic recombination at this locus. First, the asymmetries in the outside marker combinations among selected prototrophs were not a result of selection for certain flanking marker phenotypes. The parental versus recombinant and R_1 versus R_2 asymmetries are maintained in crosses made with all four possible outside marker combinations (Table 8). Second, the recombination frequencies of self crosses show that reversion and the appearance of new suppressors

TABLE 8

Distribution of flanking markers among recombinants from crosses with different arrangements of outside markers

Cross 1: $\frac{+ \quad 1+ \quad met10}{cdc14 \quad +2 \quad +}$ Cross 2: $\frac{cdc14 \quad 1+ \quad +}{+ \quad +2 \quad met10}$

Cross 3: $\frac{cdc14 \quad 1+ \quad met10}{+ \quad +2 \quad +}$ Cross 4: $\frac{+ \quad 1+ \quad +}{cdc14 \quad +2 \quad met10}$

Alleles of *SUP6*	Cross	Fraction P_1	P_2	R_1	R_2	R	Number tested
1-7 × 1-6	1	.29	.30	.24	.17	.41	296
	2	.28	.31	.26	.15	.41	289
	3	.30	.29	.25	.16	.41	297
	4	.27	.25	.30	.19	.49	287
1-7 × 1-5	1	.39	.33	.23	.05	.28	57
	2	.37	.33	.23	.07	.30	112
	3	.24	.39	.28	.09	.37	287
	4	.26	.39	.24	.11	.35	286
1-10 × 1-5	1	.26	.38	.21	.05	.36	287
	2	.18	.39	.34	.09	.43	265
	3	.26	.30	.21	.13	.34	95
	4	.25	.39	.22	.15	.37	314

did not contribute significantly to the heteroallelic crosses. The average frequency of prototroph formation for self crosses was 13×10^{-6}. This is approximately an order of magnitude lower than the heteroallelic recombination rates, with the exception of crosses *AC × 1-9* and *1-8 × 1-3*.

R_1:R_2 mapping placed alleles *1-8*, *1-10* and *1-11* in adjacent positions and because of the low frequency of recombination, they could not be resolved meiotically. With the exception of cross *1-8 × 1-3*, these three alleles behaved similarly in all crosses. The behaviour of allele *1-2* can be explained most simply if it is a deletion covering the sites of alleles *1-6*, *1-8*, *1-10* and *1-11*. Although *AC × 1-9* and *1-8 × 1-3* had low recombination frequencies, R_1:R_2 mapping placed them in nonadjacent positions.

The R_1:R_2 inequality was significant in all but 6 heteroallelic crosses (Table 6) and it is this difference which was used to order the sites. Ordering all pairs of alleles in this way gives no internal inconsistencies, and the resulting map is shown in Figure 2. Since 5 of the 6 crosses in which the inequality was not significant involved nonadjacent alleles on this map, this does not introduce any ambiguity in the map order. The remaining uncertainty concerns the relative positions of alleles *AC* and *1-3*.

Below the order in Figure 2 are shown the prototroph frequencies from two-point crosses. The frequencies do not appear to be related to the order based on flanking marker distribution, nor can they easily be used to arrange the alleles in any other sequence.

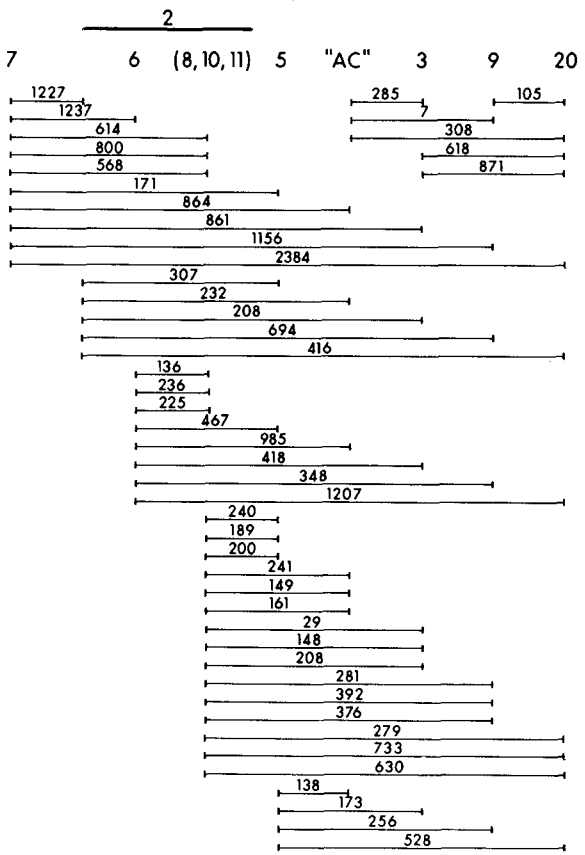

FIGURE 2.—Meiotic fine structure map of *SUP6*, with prototroph frequencies/10^6 spores.

DISCUSSION

Conversion and co-conversion in the SUP6 *region*: Results from Table 1 concerning the frequency of conversion and co-conversion in unselected tetrads are summarized in Figure 1. All four of the markers used had an unusually high conversion frequency. About 17% of all events showed co-conversion of more than one marked locus, and about 1% of conversions in this region were co-conversions of all four markers. Postmeiotic segregation of *SUP6* also frequently involved co-postmeiotic segregation of other loci. Fifteen percent of *SUP6* postmeiotic segregants extended into another locus, and 3 of the 52 postemeiotic segregations covered *his2*, *cdc14* and *SUP6*. The *his2* and *SUP6* loci are 11 map units apart on the map of MORTIMER and HAWTHORNE (1973), and *his2–met10* spans a length of 14 map units. Although it can be seen from Figure 1 that a large part of these values is derived from the conversion events themselves, so that the values do not represent normal crossover distances, the data in Table 1 make it clear that the true crossover map distances in this region are appreciable. We know of no report of conversion events of comparable lengths in other

systems, except one tetrad in Aspergillus described by STRICKLAND (1958) and the work of PASZEWSKI (1967) in Ascobolus where two spore color loci at a distance of 10 map units showed co-conversion in 1.3% of tetrads. However, there have been several reports of co-conversion involving two closely linked loci (MURRAY 1970; TOURÉ 1972).

Both the high conversion frequency of *SUP6* and the high frequency of co-conversion with other loci were found to be independent of the allele of *SUP6* which was used. Similar results were obtained when the original suppressor mutation was crossed to wild type and when two different mutated suppressors were crossed to a suppressor strain (crosses A75 and A76).

Comparison of the prototroph frequencies for *SUP6* heteroallelic crosses (Table 6 and Figure 2) with conversion frequencies in unselected tetrads (Table 2 and Figure 1) shows that the vast majority of recombination events do not stop within *SUP6*. It was found that while a conversion event covered *SUP6* in 15% to 20% of tetrads, allelic recombination was in the order of 0.04%–0.8% per tetrad. Few of the conversion events end within a marked region of the *SUP6* locus.

A high proportion of the conversion events detected do not involve *SUP6*. This suggests that there is a very high frequency of initiation of events at many sites within this region, rather than a single derepressed initiation site close to *SUP6*. This conclusion is supported by the absence of a gradient in conversion frequencies declining with distance from *SUP6*, since *his2* has a much higher conversion frequency than *cdc14*.

The conclusion from these data is, then, that the exceptionally high conversion frequency of markers in this region is caused by high frequency of initiation at several sites in or near the region, and by the extreme length of a high proportion of the events.

The tetrad data lead to a further conclusion that almost all non-crossover events involving *SUP6* concern only one chromatid. The evidence is that in 5:3 and 3:5 segregations without crossing over, three chromatids are of the parental type and one chromatid shows postmeiotic segregation (a-b, a-b, A+/—B, A+B). One never recovers the product: a-b, a+b, A+/—B, A+B. The latter would have required hybrid DNA formation on both chromatids. In addition, apparent two-strand double crossovers, which would be expected to arise from conversion in opposite directions on two chromatids, are never seen. Other yeast loci which have been analyzed show this same property (FOGEL and HURST 1967; FOGEL and MORTIMER 1969), however single chromatid events are not characteristic of recombination in all systems (LEBLON and ROSSIGNOL 1973; KITANI and WHITEHOUSE 1974).

Conversion characteristics of individual markers: Parity of conversion to mutant and to wild type was observed for all three alleles of *SUP6*, for the *his2* allele, for the *cdc14* allele and for the unlinked markers *ura4–11* and an allele of *leu2*. However, *met10–4* showed an excess of 3:1 segregations over 1:3 segregations (92:53, Table 2). Parity has been reported previously for all alleles in *Saccharomyces cerevisiae* (FOGEL, HURST and MORTIMER 1971), but LAWRENCE *et al.* (1975) published data which show a significant deviation from parity for

an allele of *met3*. The explanation for the lack of parity for *met10–4* appears to be the inviability of *met10–4* spores relative to *MET10* spores. An analysis of 900 spores from 375 incompletely viable four-spored tetrads showed that there were four times as many *met10–4* spores lost as *MET10* spores. This is sufficient to account for the observed deficiency of 1:3 segregations for *met10*.

Although Table 1 reports postmeiotic segregation for markers other than *SUP6*, sectored colonies were reliably identified only by color sectoring. For all three alleles of *SUP6*, the frequency of tetrads with postmeiotic segregation was the same at between 2% and 3%. In addition, the relative amount of aberrant segregation which was postmeiotic (9%–14%) did not differ significantly with different alleles.

Crossover and non-crossover conversion events: In contrast to the results of HURST, FOGEL and MORTIMER (1972), it was found that outside marker exchange accompanied intragenic recombination at *SUP6* significantly less than 50% of the time. Some of this discrepancy (37% outside marker recombination in tetrads from this study (Table 3) versus 51% in the study done by HURST, FOGEL and MORTIMER) can be accounted for by the use of different outside markers. Their study made use of *his2* and *met10* as flanking markers, which are 14 map units apart: *cdc14* and *met10*, used here, are separated by 10 map units (MORTIMER and HAWTHORNE 1973). The presence of *cdc14* allowed the identification of crossovers in the *his2–cdc14* region as independent of conversion of *SUP6*. If the marker *cdc14* had not been used in this study and all crossover tetrads counted as recombinant whether or not they involved the converted chromatid, no inequality between the parental and recombinant classes would have been found. STADLER (1973), in a reanalysis of the results of HURST, FOGEL and MORTIMER (1972), pointed out that when independent crossovers accompanying intragenic events are taken into account, marker exchange accompanying intragenic recombination is significantly less than 50%.

There are several ways in which a real equality of parental and recombinant classes may appear as an inequality. In the present case, however, the following considerations make it highly unlikely that the observed difference does not reflect a real inequality of the frequency of crossover and non-crossover events occurring at *SUP6*.

1. *Mutation*: In the study of random spores, the self crosses show a very low frequency of reversion to the suppressor phenotype. Thus, reversion of these secondary mutants and mutations at other suppressor loci are not the source of the excess of suppressors with parental outside markers. Furthermore, crosses of presumptive recombinant suppressors showed that 49/50 were at the *SUP6* locus.

2. *Selection for a particular outside marker combination*: Table 8 makes it clear that for random spore analysis whatever the configuration of the cross, and whether the recombinant classes were mutant for none, one or both outside markers, the frequencies in each class remained relatively constant for any one pair of alleles. Furthermore, the parental versus recombinant inequality is present in the tetrad data as well, and therefore is not a consequence of inadvertent

selection for certain combinations of outside markers when selecting for a functional suppressor.

3. *Independent crossovers, and conversions of outside markers*: In the random spore data independent crossovers and independent conversions of *cdc14* and *met10* would have the effect of transferring recombinant events into the parental class and the converse. Since the exchange goes both ways, its effect will depend on the proportions of parental and recombinant convertants. If parental exceeds recombinant, more parental events would be lost than gained, so that the true excess of parental over recombinant prototrophs would be greater than that observed.

In tetrads, one can estimate the number of unrelated crossovers which affected the converted chromatid. These should be equal to the number detected because they did not involve the converted chromatid. It will be seen from Tables 3 and 4 that there are only six such tetrads in the parental class, and seven in the recombinant class. Consequently, any correction for unrelated crossovers is small, and goes equally in the two directions.

4. *Allele specific effects*: The parental-recombinant inequality is not an allele-specific phenomenon. It is apparent in all 48 crosses involving 11 alleles of *SUP6* shown in Table 6.

5. *Selection against some types of tetrads*: Since conversion can be detected only in complete tetrads, we must consider the possibility that tetrads with three or fewer viable spores included more convertant tetrads with an associated crossover than convertant tetrads with parental flanking marker combinations. The overall frequency of incomplete tetrads was 34%. A sample of 375 of these was analyzed. Among these there was a significant deficiency of one of the parental combinations of *cdc14* and *met10*, but no significant difference between the two recombinant combinations. So crossover tetrads were not shown to be lost by a relative inviability of a recombinant genotype. In spite of this, more incomplete tetrads contained recombinant products than was expected. There are too many unknowns in the data to rule out the hypothesis that all these excess recombinant tetrads were crossover convertants of *SUP6*. We are, therefore, unable to exclude the hypothesis that the event itself (conversion of *SUP6* with crossing over) is a cause of reduced viability.

This study supports the idea that the equality or inequality of crossover and non-crossover events may be determined from selected intragenic recombinants as well as from tetrads. We have shown in Table 8 that the inequality as seen in random products is not caused by selection against particular outside marker combinations. In any case, the discussion of incomplete tetrads above makes it clear that tetrad data are also not free of such considerations. Since intragenic crossing over would give rise to intragenic recombination which would always be crossover for outside markers, some random spore data may show an excess of recombinant products which does not reflect a real difference in the proportion of events. In these data, however, the discrepancy is in the direction of an excess of parental configurations. Both unselected tetrad and selected intragenic recombination data are subject to disturbances by allele specific effects or by variation

in the proportion of crossover and non-crossover events detected within different parts of a locus. In these data we have shown, by studying intragenic recombinants involving several alleles throughout the locus (Table 1), that the excess of non-crossover events is neither allele specific nor confined to a certain part of the gene.

The above considerations lead to the conclusion that there is a real excess of non-crossover to crossover events accompanying conversion at *SUP6*. The results also show that this effect is specific to the immediate region of *SUP6*, since *cdc14* convertants and *SUP6–cdc14* co-convertants do not show this excess.

WHITEHOUSE (1974), in his analysis of recombination data for the *g* locus of *Sordaria fimicola*, offered mechanisms by which an inequality between crossover and non-crossover events would be observed without altering the basic assumption that a random decision is made, as described by SIGAL and ALBERTS (1972). The particular deviation seen here, an excess of non-crossover events, is explained by postulating that when a correction length reaches the end of a heteroduplex length, the event is always resolved into a non-crossover. One may test this by looking at events in which correction has not taken place, that is postmeiotic segregations. In this study, the frequency of outside marker recombination in chromatids showing postmeiotic segregation was 46%. This is 10% higher than the amount of reciprocal recombination associated with conversion. While the bias is in the expected direction, this sample of tetrads in which correction was not involved is small and the difference is not significant.

Mappability and polarity at the SUP6 *locus*: The results in Table 7 and Figure 2 show *SUP6* to be mappable by the inequality of R_1 and R_2 classes among intragenic recombinants. The near equality of parental classes indicates that there is no polarity at this locus. The formation of intragenic recombinants shows no clear distance dependence. The results in Figure 2 also show no evidence of map expansion.

This locus is also immappable by the technique of gamma ray induction of mitotic recombination (DICAPRIO, unpublished results). Although a linear dose response is found in any one experiment, there is considerable variability in the slope obtained from different determinations using the same diploid. Analysis of prototrophs obtained by gamma irradiation of heteroallelic diploids shows that many carry new suppressor mutations, and most of the rest arise by complex intragenic events which show long lengths of co-conversion similar to those seen among meiotic events.

The immappability of *SUP6* by recombination frequencies is not a unique situation in meiotic fine structure mapping. The *mtr* locus of *Neurospora* (STADLER and KARIYA 1969) is immappable by either prototroph frequency or the R_1:R_2 inequality, as is *am-1* (FINCHAM 1967). The *am-1* data are interesting in that immappability is accompanied by strong polarity unlike the situation described here (FINCHAM 1974). KRUSZEWSKA and GAJEWSKI (1967) reported that in the *Y* locus of *Ascobolus immersus* the frequency of recombination is determined by the basic conversion frequency of each allele and is not dependent on the distance between the alleles.

While most fine structure mapping in *Saccharomyces cerevisiae* has made use of induced mitotic recombination, the few published results on meiotic prototroph frequencies generally show that alleles can be ordered by this method, but with large deviations from additivity (FOGEL and HURST 1967; JONES 1972; ESPOSITO 1968). ROTHSTEIN (1975) has constructed a meiotic fine structure map of *SUP3*, and while there are again large discrepencies, the alleles can be ordered using prototroph frequencies, which indicates that immappability is not a general characteristic of suppressors in *Saccharomyces*.

MOORE and SHERMAN (1975) devote an extensive discussion to examples where physical distance and sometimes allele order are not reflected in the genetic map. In their study of recombination between alleles of the cytochrome *c* gene where physical distances are known, they found that spontaneous and induced mitotic recombination and meiotic recombination all showed large discrepancies between physical and genetic lengths, and none could be used reliably to order the alleles. The discrepancies appear to be a result of complex interactions of nucleotide sequence, and the patterns are different in meiotic and mitotic methods.

Although MOORE and SHERMAN (1975) did not include the R_1 versus R_2 method in their analysis, this technique does not appear to be independent of the others since it is thought to be based on some of the same parameters. So the results of using this method should also be treated with caution.

General considerations: Against the general observation that the frequency of recombination events is under locus-specific control (HASTINGS, 1975), probably an operator system, PUTRAMENT (1971) has suggested that the local structure of the chromatin is responsible for this control. In these data we have described a region of a chromosome in which a cluster of several initiation sites shows a frequency of events an order of magnitude higher than that experienced in most regions in the same organism.

The high frequency of events is correlated with an average length of conversion events which is at least as aberrant as the frequency of events. Since a clustering of high frequency initiation sites would not explain the long conversion lengths, it is easier to suppose that this correlation of extremes of two parameters over an extended region is attributable to the general chromosome structure in that region, as suggested by PUTRAMENT, than it is to postulate that a number of operator-like control regions with similar properties are clustered together.

Locus-specific aspects of control are still suggested, however, by the variation in conversion frequencies at different loci within the cluster, and by the specific occurrence of the inequality between parental and recombinant events. So we suggest that both regional and local controls of recombination are operating.

Because of the difficulty of determining the relative position of mutant sites in a region with very long conversion lengths, this system appears to be unsuitable for studies which correlate the physical structure of a tRNA with genetic data. It is, however, suitable for studies in which the frequency of conversion and post-meiotic segregation are to be measured, such as that of LEBLON and ROSSIGNOL (1973).

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