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A *buff* spore colour mutant in *Sordaria brevicollis* showing high-frequency conversion

2. Loss of the high-frequency conversion

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SUMMARY

A mutant, YS17, at the *buff* spore colour locus in *Sordaria brevicollis* has previously been described. It shows conversion with high frequency, predominantly to wild type, and is believed to act as a recognition site for an endonuclease that initiates recombination at the YS17 site. The discovery is now reported of a gene that causes loss of the high-frequency conversion shown by the YS17 mutant. The gene was present in existing stocks of the fungus. It reduces the conversion frequency of YS17 to a level similar to that of other *buff* mutants, from which it is inferred that the YS17 mutant no longer acts as an initiation site for recombination. When the conversion frequency of YS17 is low the bias in conversion to wild type rather than to mutant is lost, suggesting that this bias may relate to the initiation of recombination at the site. The loss of high frequency conversion of YS17 appears to be determined by a single recessive gene linked to mating type and unlinked to *buff*. It is suggested that the dominant allele induces recombination at the site of YS17 by controlling either the synthesis or the activity of an endonuclease that is capable of recognising the nucleotide sequence at the YS17 site. Some anomalous results point to the existence of modifiers of the action of the gene.

INTRODUCTION

The *Sordaria brevicollis buff* mutant YS17 had been obtained by Dr C. Yu-Sun (see MacDonald & Whitehouse, 1979) by treatment of microconidia with ICR170 and plating on a 5-day-old culture of the wild type of the other mating type bearing protoperithecia. The mutant was detected by the presence of a cluster of spores, half of which were pale, shot from an individual perithecium onto the lid of the petri dish. Isolates of YS17 of each mating-type were obtained and crossed with wild type. These initial crosses showed conversion of YS17 to wild type with a frequency of *c.* 11% (see Table 1 of MacDonald & Whitehouse, 1979).

Evidence from four sources was in agreement with the hypothesis that the YS17 mutation is a recognition site for an endonuclease that initiates recombination (MacDonald & Whitehouse, 1979). (i) The frequency of aberrant segregation at the site of YS17 is an order of magnitude greater than for other *buff* mutants, in crosses

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with wild type. (ii) The pattern of polarity in recombination within the *buff* gene is modified in the presence of YS17, conversion at its site exceeding that of alleles in two-point crosses, whether they map on its proximal or distal side. (iii) An attempt to separate the YS17 mutation causing *buff* spore colour from that causing high frequency conversion failed. (iv) The frequency of postmeiotic segregation at the sites of *buff* mutants near to the site of YS17 is increased in the presence of YS17 and occurs in the chromatid showing conversion to wild type at YS17.

The occurrence is now described of the loss of the high-frequency conversion previously shown by the YS17 mutant.

MATERIAL AND METHODS

The mutants used, the culture media and cultural conditions for growing the mutants, and the techniques for handling the organism were described previously (MacDonald & Whitehouse, 1979).

RESULTS

Discovery of loss of high frequency conversion of YS17

Loss of the elevated conversion frequency of YS17 was discovered by one of us (M.V.M.) some months after YS17 had been isolated. Crosses of YS17 with alleles gave frequencies of recombinant asci ranging from 0·15 to 12·1 % depending on the *buff* mutant (see Table 4 of MacDonald & Whitehouse, 1979). When YS17 of *A* mating type was crossed with *buff* mutant S156 of *a* mating type the frequency of recombinant asci was 9·1 ± 0·4 %, but the reciprocal cross (S156*A* × YS17*a*) gave no recombinants in 5965 asci scored. A similar finding was made when YS17 was crossed with another *buff* mutant, S180, the frequencies of recombinant asci in samples numbering about 6000 from YS17*A* × S180*a* and S180*A* × YS17*a* being 10·1 and 0·19 % respectively. Subsequently, two other *buff* mutants, YS9 and YS14, were found to show the same effect. In all four cases the frequency of recombinant asci was *c.* 10 % with YS17*A* and 0–0·15 % with YS17*a*.

It appeared as if certain *buff* isolates of *A* mating type carried a gene that caused loss of the high frequency conversion normally shown by YS17.

Genetic basis of the loss of high frequency conversion

The S156*A* and S180*A* isolates that showed the low frequency of recombination when crossed with YS17*a* were crossed with wild type *a*, and *buff* progeny were isolated. It was found that those of *A* mating type showed a low recombination frequency when crossed with YS17*a*, like the parent S156*A* and S180*A* strains. A total of four S156*A* and six S180*A* progeny were tested. The results are given in Table 1. On the other hand the S156 and S180 progeny of *a* mating type from the same cross showed a high recombination frequency when tested against YS17*A*. The results are also given in Table 1. The difference in frequency according to mating type was about 100-fold, as with the parents.

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It appeared as if a gene linked to mating-type was segregating causing loss of the high frequency conversion shown by YS17. Crosses were carried out in order to establish whether the gene was dominant or recessive.

Table 1. *Frequency of recombinant asci in crosses of S156 and S180 with YS17, when the S156 and S180 isolates were obtained from crosses of the original S156*A *and S180*A *strains with wild type* a

Mating type of S156 or S180 isolate	Buff mutant crossed with YS17	Isolate number	Total number of asci examined	Number of recombinant asci with wild type spores	Percentage frequency of recombinant asci
A	S156	2	2137	2	0·09
		6	2087	0	0
		8	2013	0	0
		10	2208	2	0·09
	S180	2	1797	0	0
		3	2081	6	0·3
		5	1554	1	0·06
		6	786	0	0
		7	2089	1	0·05
		8	2288	0	0
a	S156	1	2569	176	6·9
		3	2174	127	5·8
		4	2031	115	5·7
		5	2457	185	7·5
		7	1621	109	6·7
		9	1990	141	7·1
	S180	1	2171	216	9·9
		4	2096	150	7·2
		9	2191	219	10·0
		10	2132	243	11·4

Hypothesis of a dominant gene for loss of elevated recombination

The parental *YS17a* showed low values when crossed with the parental *S180A* and *S156A*, but it showed a high value when crossed with the parental *wtA*.* It follows that if the low value is due to a dominant gene it must be present in *S180A* and *S156A* and not in *YS17a*. This conclusion is supported by the fact that the *S180A* and *S156A* testers consistently gave low values with all the YS17 isolates with which they were crossed (Table 2), apart from a few exceptions. Conversely, the *YS17a* tester gave diverse results (Tables 3 and 4), high with some crosses and lower with others. The gene is evidently closely linked to the mating-type locus. This is shown, in particular, by crosses 7, 8, 12 and 13, where the *A* progeny gave low values consistently and the *a* progeny gave high values, also consistently (Tables 3 and 4). There is one result, however, that conflicts with the dominant

* In this and the following section parental strains are given in italics.

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hypothesis: the wt*A* progeny from cross 12 evidently carried the gene (as expected from their parentage) as they gave low values when tested with the parental *YS17a* tester (Table 4); the YS17*A* progeny, however, from the latter cross (no. 16) gave high values when tested against the parental *S156a* (Table 2). But this result is consistent with the hypothesis that the gene for low values is recessive, if *S156a* carries the dominant allele for high conversion frequency.

Table 2. *Results with S156, S180 and wt as testers*

(Parental strains are in *italics*. H and L indicate high and low recombination frequencies, respectively.)

Results with tester strains						Cross and progeny (mating-type)			Results with tester strains					
wta		*S156a*		*S180a*					*S156A*		*S180A*		*wtA*	
H	L	H	L	H	L	*A*		*a*	H	L	H	L	H	L
H	—	H	—	H	—	*YS17*	1	*YS17*	—	L	—	L	H	—
		3	0	3	0	YS17 ↙		↘ YS17	1	6	1	6		
						wt	2	*YS17*	—	L	—	L	H	—
		3	0	3	0	YS17 ↙		↘ YS17	0	6	0	6		
H	—	H	—	H	—	*YS17*	3	*wt*						
		6	0	6	0	YS17 ↙		↘ YS17	1	2	1	2		
						wt*	16	*YS17*	—	L	—	L	H	—
0	11	15	0			YS17 ↙		↘ YS17	0	10			25	0

* From cross no. 12 (Table 4).

Recessive hypothesis

The implication of this hypothesis is, of course, that whenever a low value is obtained both parents carry the gene. It follows that the parental *YS17a* must carry it as well as the parental *S180A* and *S156A*. It also follows that the parental *wtA* does not carry it, since it gave a high value with *YS17a*. Since the gene is closely linked to mating-type (see, for instance, cross 2 in Table 2) it is evident from cross 3 (Table 2) that the parental *wta* must carry it, in order to account for the low value shown by the YS17*a* progeny in cross 3 when tested against *S156A* and *S180A* (though one of the progeny gave a high value). If the parental *wta* carries the gene for low values it follows that *YS17A* does not, since the cross of these two gave a high value. The wt*A* progeny from cross 12 evidently carry the gene (as expected from their parentage), since they gave a low value with *YS17a*

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(Table 4). The YS17*A* progeny derived in cross 16 from these wt*A* isolates would also carry the gene, and this was confirmed by the low value when tested against the parental *wta* (Table 2). But when tested against the parental *S156a* they gave a high value (as already mentioned in discussing the dominant hypothesis). It follows that *S156a* does not carry the gene. The data in Tables 1–3 seem to be consistent with a recessive gene for low values linked to mating-type and present initially in *S180A*, *S156A*, *YS17a* and *wta*, but not detected in the other parental cultures. There are, however, a number of exceptional progeny not expected on this hypothesis (see Discussion). Nevertheless it is concluded that the gene for low values is recessive.

Conversion pattern of YS17 when high-frequency conversion lost

The conversion pattern shown by *buff* spore colour mutant YS17 has been investigated in the presence and in the absence of homozygosity for the unlinked gene giving loss of high frequency conversion. From crosses with wild-type the results given in Table 5 have been obtained.

It is evident that the gene, when homozygous, not only reduces the frequency of aberrant asci (from 8·6 to 0·14 %) but also changes the pattern. In particular, the proportion of aberrant asci showing postmeiotic segregation (5:3 and 3:5 ratios) rises from 0·3 to 15 %. Previous studies with mutants in other spore-colour genes (Yu-Sun, Wickramaratne & Whitehouse, 1977) have suggested that postmeiotic segregation frequencies of 0–8 % are shown by frameshift mutants and higher values (14–54 %) by substitution mutants. It seems possible that YS17 arose by base substitution. The high frequency conversion to wild type which it can show would then not imply that it was a deletion frameshift but would relate to its action as an initiation site for recombination. As already pointed out (MacDonald & Whitehouse, 1979) there is support for this idea from the behaviour of the *ade6* mutant M26 in *Schizosaccharomyces pombe* reported by Gutz (1971). He found that this mutant was suppressed by a super-suppressor and therefore was a polypeptide-termination mutant, with the implication that it arose by base-substitution, the remainder of the gene being translated in the normal reading-frame in the presence of the super-suppressor. When crossed with wild type the M26 mutant, like YS17, showed high frequency conversion to wild type. This was exceptional behaviour for a mutant of *S. pombe*, where conversion to wild type and to mutant are usually equally frequent. With both M26 and YS17, however, it may be that mismatch correction (if that is the cause of conversion) occurs equally often in each direction, the bias in spore genotype in favour of wild type arising because the wild type molecule functions as the donor of a strand to the mutant one much more often than the converse situation. Mismatch correction in favour of mutant would then restore a normal segregation pattern (2 wild type and 2 mutant products of meiosis) which would not be distinguishable from the numerous asci without recombination at the *ade6* or *buff* loci.

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Table 3. *Results for S180 with YS17A and a as testers*

(Parental strains are in *italics*. Figures in parentheses are spore-pairs from ascus dissection. H high, L low recombination frequency.)

Results with tester strain *YS17a* H	Results with tester strain *YS17a* L	Cross and progeny (mating-type) *A*	Cross	Cross and progeny (mating-type) *a*	Results with tester strain *YS17A* H	Results with tester strain *YS17A* L
—	L	*S180*	4	*S180*	H	—
0	2	S180		S180	8	0
H	—	*wt*	5	*S180*	H	—
				S180	4	0
3 and 1 heterogeneous§	0	S180	6	*wt*	H	—
7 and 1 medium§	4	S180		S180	15 and 2 medium§	0
—	L	*S180*	7	*wt*	H	—
0	31 + (5)	S180		S180	41 + (4)	0
—	L	*S180*	8	wt‡	31 + (21)	0
0	7	S180		S180	3	0
0	26 + (8)	wt‡	9	*S180*	H	—
0	433	S180		S180	504	2
0	5	wt*	15	*S180*	H	—
0	86	S180		S180	86	0
H	—	*wt*	17	*wt*	H	—
		wt	18	*S180*	H	—
3	0	S180		S180	13	0

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Table 3. (*cont.*)

Results with tester strain *YS17a* H	L	Cross and progeny (mating-type) *A*		*a*	Results with tester strain *YS17A* H	L
—	L	*S180*	19	wt†		
0	5	S180		S180	10	0

* From cross no. 12 (Table 4).
† From cross no. 17.
‡ From cross no. 7.
§ The heterogeneous culture (cross 5) had some perithecia with high and some with low values, and so may have been heterokaryotic. The cause of the medium values in crosses 6 and 11 was not investigated but may also have been due to heterokaryosis, or to the effect of modifying genes or developmental factors.

DISCUSSION

The repressors of recombination in *Neurospora crassa* described by Catcheside and associates (see Catcheside, 1977) differ from the situation described here. The *Neurospora* repressors are controlled by dominant genes, the higher frequency of recombination occurring when the recessive allele is homozygous. The loss of high frequency recombination in the *buff* gene of *Sordaria brevicollis*, on the other hand, is evidently controlled by a recessive gene. The *Neurospora* repressors are believed to block the activity at specific sites of an endonuclease that initiates recombination. With the gene affecting *S. brevicollis* recombination, however, the dominant allele evidently induces recombination. It might control the synthesis or the action of the endonuclease that recognises the YS17 site. Thus, this recombination locus might be the structural gene for the endonuclease, or it might specify a protein that induces the activity of the gene or allows the enzyme to become active. Such regulation could occur through interaction with the enzyme or with the *buff* gene, for example, by altering the chromatin such that the DNA at the YS17 site became accessible to the endonuclease. The anomalous results occasionally found (see Table 3, cross 9, and Table 4, crosses 10 and 14) suggest that the activity of the recombination gene is susceptible to modification by genetic or developmental factors. It is perhaps easiest to understand this modification if the recombination gene regulates the activity of the endonuclease rather than its synthesis.

The effect of the gene causing loss of YS17 high-frequency conversion on recombination in spore colour genes other than *buff* has been investigated, as this should provide a better understanding of its action. The results obtained will be discussed in a later paper, together with linkage data for the gene and the spore colour mutants.

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Table 4. *Results for S156 with YS17A and a as testers*

(Parental strains are in *italics*. H high, L low recombination frequency.)

Results with tester strain *YS17a*		Cross and progeny (mating-type)			Results with tester strain *YS17A*	
H	L	*A*	Cross	*a*	H	L
—	L	*S156*	10	*S156*	H	—
2	1	S156		S156	7	0
H	—	*wt*	11	*S156*	H	—
6	0	S156		S156	2 and 1 medium‡	0
—	L	*S156*	12	*wt*	H	—
0	4	S156		S156	6	0
—	L	*S156*	13	wt*	3	0
0	23	S156		S156	6	0
0	5	wt*	14	*S156*	H	—
15	9	S156		S156	18	2
H	—	*wt*	17	*wt*	H	—
		wt	20	*S156*	H	—
6	0	S156		S156	9	0
—	L	*S156*	21	wt†		
0	7	S156		S156	7	0

* From cross no. 12.
† From cross no. 17.
‡ See footnote § of Table 3.

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Table 5. *Numbers of asci in crosses of YS17 and wild type*

Conversion frequency	Wild-type : mutant spores in ascus						Total
	4:4	6:2	2:6	5:3	3:5	8:0	
Low	30722	17	16	2	6	1	30764
High	53936	4999	9	14	1	47	59006

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REFERENCES

Catcheside, D. G. (1977). *The Genetics of Recombination*. London: Arnold.

Gutz, H. (1971). Site specific induction of gene conversion in *Schizosaccharomyces pombe*. *Genetics* **69**, 317–337.

MacDonald, M. V. & Whitehouse, H. L. K. (1979). A *buff* spore colour mutant in *Sordaria brevicollis* showing high-frequency conversion. I. Characteristics of the mutant. *Genetical Research* **34**, 87–119.

Yu-Sun, C. C. & Wickramaratne, M. R. T. & Whitehouse, H. L. K. (1977). Mutagen specificity in conversion pattern in *Sordaria brevicollis*. *Genetical Research* **29**, 65–81.

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