GENE CONVERSION OF CYSTEINE MUTANTS IN NEUROSPORA[1]

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SEVERAL cases of the following type have been reported in studies of microorganisms in recent years: two or more mutants of independent origin have similar phenotypes and appear to be allelic to each other or very closely linked; crosses between the mutants yield a small proportion of nonmutant progeny, but the event producing these recombinants does not have the characteristics of a crossover. Such a case, involving two cysteine-requiring mutants of *Neurospora crassa*, is reported here.

METHODS

Crosses were made at 25°C on synthetic crossing medium (WESTERGAARD and MITCHELL 1947) supplemented with the growth factors required by the protoperithecial parent (250 mg/liter of cysteine and in some cases 50 mg/liter of adenine or of lysine). To isolate cysteine-independent progeny, ascospores were picked up from the wall of the cross tube in loopfuls of water and spread on petri plates of Fries minimal medium (BEADLE and TATUM 1945) without cysteine, but supplemented to be nonselective for linked markers; most plates contained less than 500 ripe spores. Plates were heat shocked and then incubated at 25°C. Starting 15 hours after heat shock, plates were examined at intervals for the next 24 hours, and all cysteine-independent colonies were transferred to slants of complete medium to classify for the linked markers. Total numbers of germinated spores were determined by direct counts on the same plates.

Forty-two of the cysteine-independent strains were crossed to wild type to confirm that their genotypes corresponded to the vegetative phenotypes. Analyses of asci from these crosses revealed in every case that vegetative classification had been correct. This was a check against misclassification of linked markers and also against the possibility of the *cys*+ condition arising from a pseudo-wild type (MITCHELL, PITTENGER and MITCHELL 1952) rather than a true *cys*+ genetic region.

EXPERIMENTAL RESULTS

Strains 80702 and 48401 are single gene cysteine-requiring mutants of Neurospora. They differ in nutritional responses at 25°C. 80702 (hereafter referred to as *cys-t*) will grow on minimal medium supplemented with either thiosulfate or cysteine, while 48401 (*cys-c*) will grow on cysteine but not on thiosulfate. (At

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35°C both mutants respond slightly to thiosulfate.) These mutants do not appear to complement each other in heterocaryons. A cysteine-independent heterocaryon made up of strains carrying the two mutants has not been obtained. A heterocaryon of such strains, "forced" by other auxotrophic mutants present in the component strains, required cysteine for growth.

Both mutants are known to be in linkage group VI (M. FLING, unpublished). A cross between them yields about 0.2 percent cysteine-independent progeny. Crosses of *cys-c* × *cys-c* or *cys-t* × *cys-t* yield no *cys*+ progeny. If the *cys*+ spores from the cross between the two mutants resulted from crossing over, it would mean that the two cysteine loci were 0.4 map units apart. However, the *cys*+ progeny appear to result not from crossing over, but rather from gene conversion (MITCHELL 1955). (In this report, the term "gene conversion" is used to mean recombination between closely linked or allelic mutant genes by a process not having the characteristics of crossing over; the use of the term does not imply any specific mechanism for the event.)

Evidence that cys+ *spores do not result from crossing over:* The cysteine region of group VI lies between the loci of the mutants *un* and *ylo* (STADLER 1956). *un* is a temperature mutant which grows on minimal medium at 25°C but has an unknown requirement at 35°C; *ylo* is a visible mutant with yellow conidia instead of the wild type orange. The cross *un cys-c ylo*+ × *un*+ *cys-t ylo* yielded 137 *cys*+ progeny among 72,545 germinated spores. The total recombination frequency between *un* and *ylo* in this cross was 7.7 percent. If the cysteine-independent spores had arisen by crossing over between two separate cysteine loci, the majority of them might have been expected to be one recombinant type for the outside markers (either *un cys*+ *ylo* or *un*+ *cys*+ *ylo*+). Instead, all four possible combinations of outside markers occurred with appreciable frequencies among the *cys*+ progeny (Table 1).

Asci of the above cross were dissected into spore pairs and examined to determine whether the event yielding a *cys*+ spore pair also gave a complementary product (a cysteine double mutant) as would be expected from crossing over. A total of 153 asci were dissected in which all four spore pairs germinated; these included three asci each containing one *cys*+ spore pair (Table 2). The nine cysteine-requiring strains from these asci were all checked for the double mutant

TABLE 1

Cys+ *progeny of the cross* un cys-c ylo+ A × un+ cys-t ylo a

Crossover map distances: *un* 2.1 *cys* 5.6 *ylo*
Cys+ spores among 72,545 germinated random spores:

68	*un cys*+ *ylo*+
26	*un*+ *cys*+ *ylo*
18	*un cys*+ *ylo*
25	*un*+ *cys*+ *ylo*+
137	TOTAL

TABLE 2

Asci containing cys+ *progeny*

un	*cys**	*ylo*	Mating type	Backcross test (Production of *cys*+ progeny in cross to) *cys-c*	*cys-t*
+	+	+	a		
un	*cys-c*	+	a	—	+
un	*cys-t*	*ylo*	A	+	—
+	*cys-t*	*ylo*	A	+	—
un	*cys-c*	+	A	—	+
un	*cys-c*	+	A	—	+
+	*cys-t*	*ylo*	a	+	—
+	+	*ylo*	a		
+	+	*ylo*	A		
+	*cys-t*	*ylo*	A	+	—
un	*cys-c*	+	a	—	+
un	*cys-c*	+	a	—	+

* The *cys* classifications in this column are based on nutritional tests at 25°C.

condition by the backcross test of MITCHELL (1955). (The basis of this test is the assumption that a cysteine double mutant will fail to give *cys*+ convertants in crosses to either parental mutant). Each of the nine proved to be one or the other of the parental single mutant types (Table 2).

Relationship of gene conversion to crossing over: The event yielding *cys*+ progeny does not have the characteristics of classical crossing over, but it does show a correlation with recombination between *un* and *ylo*. Only 7.7 percent of the spores in the general population are recombinants for *un* and *ylo*, while 43 of the 137 *cys*+ spores are recombinants for these markers (Table 1).

In Neurospora, different crosses segregating for the same markers frequently show pronounced differences in recombination frequency (STADLER 1956). Four more crosses of *cys-c* × *cys-t* have been studied in which *un* and *ylo* were also segregating. The aim was to learn whether changes in the frequency of crossing over would result in any consistent change in the frequency or type of *cys*+ conversion.

In Table 3 the five *cys-c* × *cys-t* crosses are arranged in order of increasing recombination frequency for the *un-cys-ylo* region. From cross 1 to cross 5 there is a threefold increase in recombination. There is no corresponding increase in conversion frequency; in fact, the total frequency of conversion to *cys*+ shows only slight changes in any of the five crosses.

When the *cys*+ progeny are separated into the four different outside marker combinations, it is seen that the parental combination of markers which came into the cross with *cys-c* is consistently more frequent than the *cys-t* marker combination. Among *cys*+ progeny with nonparental combinations of *un* and *ylo*, those with the left-hand marker from the *cys-t* parent and the right-hand marker from *cys-c* are consistently more frequent than the reverse combination. But the

TABLE 3

Crossing over and gene conversion in the cys *region*

—*lys*—*un*—*cys*—*ylo*—*ad*—centromere—

Cross	Map distance†				Cys+ per 10^4 germinated spores* (actual numbers in brackets)				
	lys -un	*un- cys*	*cys- ylo*	*ylo -ad*	c+c	t+t	c+t	t+c	Total
1	..	2.8	3.8	3.8	16.4 (40)	1.6 (4)	0.4 (1)	10.0 (25)	28.6 (70 in 24,435)
2	..	2.1	5.6	..	9.4 (68)	3.6 (26)	2.5 (18)	3.4 (25)	18.9 (137 in 72,545)
3	1.2	4.9	6.2	..	13.6 (28)	1.4 (3)	2.9 (6)	5.8 (12)	23.7 (49 in 20,634)
4	..	8.1	13.0	10.8	6.8 (25)	4.1 (15)	0.8 (3)	6.2 (23)	17.9 (66 in 36,781)
5	..	5.1	17.5	10.7	9.0 (29)	2.5 (8)	0.3 (1)	10.6 (34)	22.4 (72 in 32,059)

cross 1: *un cys-c ylo+ ad+ 7A* × *un+ cys-t ylo ad 13a*
cross 2: *un cys-c ylo+ A* × *un+ cys-t ylo a*
cross 3: *lys+ un cys-c ylo A* × *lys un+ cys-t ylo+ a*
cross 4: *un cys-c ylo+ ad+ A* × *un+ cys-t ylo ad 3a*
cross 5: *un cys-c ylo+ ad+ A* × *un+ cys-t ylo ad 32a*

* *cys+* progeny are here classed according to which of the parent strains provided the linked markers immediately around the cysteine locus. For example, "c + t" designates all *cys+* spores which carry the left-hand marker from the *cys-c* parent and the right-hand marker from the *cys-t* parent.
† Map distances are based on the classification of about 200 random spores per cross.

fluctuations of these frequencies show no apparent correlation to recombination frequency.

The length of the region of interaction: While conversion to *cys+* shows a correlation with recombination for neighboring markers, it is not the product of a simple reciprocal exchange between two separate cysteine loci; thus the recombination events taking place in this region must be more complex. It would be of interest to know whether these coincident recombination events take place at a single point or whether the events extend any detectable distance along the chromosomes. Let us call that localized section of the chromosome pair which may be the site of recombination events (either conversion or crossing over or both) the "region of interaction". We should like to determine the length of this region.

In crosses 1, 3, 4, and 5 there are two segregating markers on the same side of *cys*. (The lysine mutant in cross 3, *DS6-85*, is allelic or closely linked to *asco*, a lysine-requiring mutant which arrests ascospore maturation; spores carrying *DS6-85* mature normally. The adenine mutant in crosses 1, 4, and 5 is the one known as *ad-1*.) These crosses offer a method of measuring the length of the region of interaction.

If linked markers A and B are segregating in a cross between the two cysteine mutants, and if the order of the loci is A-B-*cys*, we may be able to compare the length of the region of interaction with the B-*cys* distance. We will compare the

A-B recombination frequency among the general population to that among *cys*+ convertants. If a region of interaction were too short to account for both events, A-B recombinants should be no more frequent among *cys*+ convertants than among random spores (and perhaps less frequent, as a result of chromosome interference). If the region of interaction were long enough to encompass the B-*cys* distance, we might expect a higher frequency of A-B recombinants among convertants than among random spores.

The frequency of *ylo-ad* recombination among *cys*+ convertants in crosses 1, 4, and 5 is not higher than the frequency of *ylo-ad* recombination in the general population (Table 4). There is thus no evidence that regions of interaction in these crosses are long enough to include the *cys-ylo* interval. Likewise, the results from cross 3 do not indicate that the region of interaction is longer than the *un-cys* distance. A similar result was reported by FREESE (1957a) in crosses between para-aminobenzoic acid requiring mutants of Neurospora. There was no correlation between conversion to *pab*+ and crossing over in nearby marked regions.

DISCUSSION

A correlation between gene conversion and recombination for linked markers has been demonstrated in several studies (for example: MITCHELL 1955; ST. LAWRENCE 1956; FREESE 1957a, b; CASE and GILES 1958; ROMAN and JACOB 1958), and two types of hypotheses have emerged regarding the relationship of gene conversion to crossing over:

TABLE 4

Relationship of cys+ *conversion to recombination in region linked to* cys *but not adjacent to it*

—*lys*—*un*—*cys*—*ylo*—*ad*—centromere—			
	un cys-c ylo+ *ad*+ × *un*+ *cys-t ylo ad*		
		ylo-ad recombination	
Cross	*cys-ylo* recombination	in general population	among *cys*+ convertants
1	2.8%	8/212 (3.8%)	2/70 (1 + + + *ad*, 1 *un* + *ylo* +)
4	8.1%	20/185 (10.8%)	5/66 (3 + + + *ad*, 1 *un* + *ylo* +, 1 + + *ylo* +)
5	5.1%	19/177 (10.7%)	1/72 (+ + + *ad*)
	lys+ *un cys-c ylo* × *lys un*+ *cys-t ylo*+		
		lys-un recombination	
Cross	*un-cys* recombination	in general population	among *cys*+ convertants
3	4.9%	2/162 (1.2%)	1/49 (*lys un* + *ylo*)

Separate events: MITCHELL (1955) proposed that crossing over and conversion were separate events but that both might be favored by an especially intimate pairing of the duplicating strands. If the degree of association were not uniform along the length of a pair of homologous chromosomes, then the two types of recombination event would show a correlation in regions of close pairing. It has been suggested (TAYLOR 1957; ROMAN and JACOB 1958) that crossing over might represent exchange along the protein chromosome backbone, while conversion takes place along DNA side chains.

Single event: FREESE (1957b) has proposed a "switch hypothesis" by which the same event may result in both conversion and outside marker recombination. "Within a region of intimate pairing two new chromosomal strands duplicate partially along one parental strand, partially along the other, switching forth and back from one information source to the other." The number of switches may be two or three or more, thus randomizing outside marker combinations. The two strands are not required to make exactly reciprocal switches; this would account for the absence of the complementary product of conversion (Figure 1).

Crosses with markedly different recombination frequencies in the region around the cysteine locus do not show corresponding differences in the frequency of gene conversion. This finding does not lend strong support to either the single

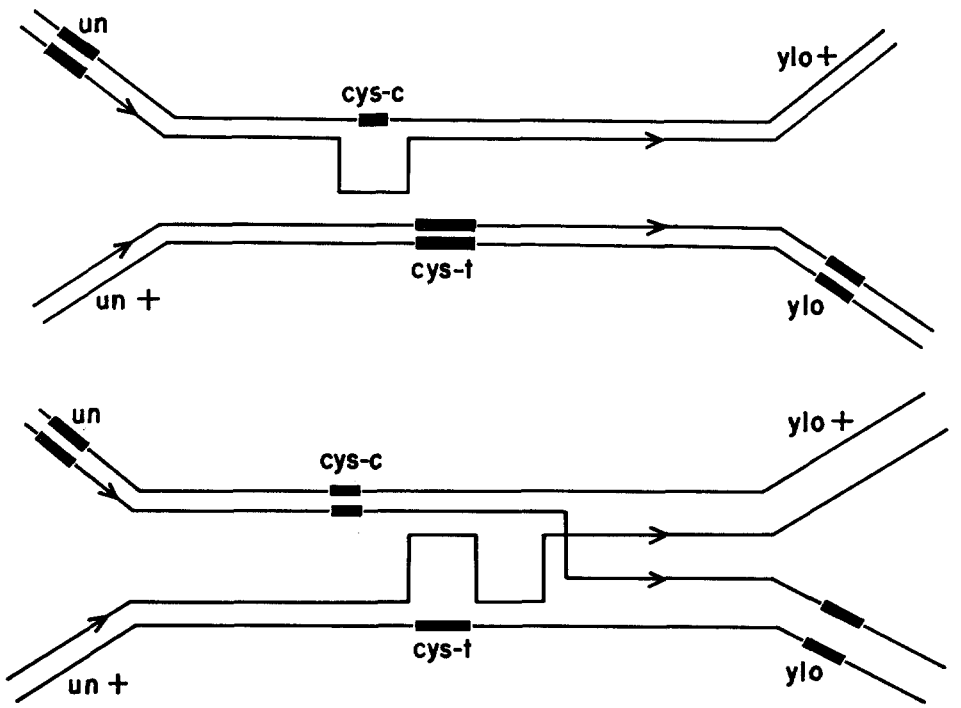

FIGURE 1.—Gene conversion at the cysteine locus by the switch hypothesis. Above: conversion of *cys-c* to *cys* + without linked marker recombination. Below: conversion of *cys-t* to *cys* + with marker recombination.

event or the separate events hypothesis. Perhaps any such "separation" of the two types of recombination argues for their being separate events. However, results somewhat different from these might be predicted from the separate events hypothesis in its simplest form. A constant rate of gene conversion with different recombination frequencies might be expected, but one would predict that increased recombination would shift conversion products with parental combinations of markers to those with nonparental combinations. No such trend is evident from the data.

One aspect of the results which appears to put constraint on the single event hypothesis of FREESE is the finding of a consistent inequality in the numbers of the two types of convertants with parental combinations of adjacent markers; in every cross the *cys-c* combination occurred more frequently than the *cys-t* combination (Table 3). According to the switch hypothesis either of these types could result from a double switch, and there seemed no *a priori* reason to expect the event to embrace one of the mutant genes more often than the other. It might be argued, however, that the length of the mutant region in *cys-t* is similar to the distance between successive switches in a switch region, while the mutant region of *cys-c* is shorter. In such a situation, only a small fraction of the double switches would be so placed as to completely extirpate the mutant region of *cys-t*, while the removal of *cys-c* would be accomplished more often (see Figure 1).

The experiment designed to measure the length of the region of interaction offers no basis of choice between the single event and the separate events hypotheses. The region of interaction by the switching hypothesis would be the region of switching, while the region of interaction for the separate events hypothesis might be the region of especially close pairing. The results observed here serve only to put an upper limit on the length of this region.

Among *cys*+ convertants with nonparental combinations of markers at the *un* and *ylo* loci, all five crosses yielded more with the left-hand marker from the *cys-t* parent and the right-hand marker from the *cys-c* parent than the reverse combination. Such a disparity might be explained in at least two ways:

1) The two cysteine mutant genes might be arranged along the linear axis of the chromosome in the order *un—cys-c—cys-t—ylo*, so that a single exchange could yield a *cys*+ product with the more frequent marker combination, while a more complicated event would be required to yield the reverse combination. Such a linear separation of mutant genes interacting in gene conversion is decisively demonstrated in the study of pantothenic acid mutants of Neurospora of CASE and GILES (1958). A linear arrangement of the mutant regions is implicit in the switch hypothesis, but not necessarily in the separate events scheme (MITCHELL 1955, ROMAN and JACOB 1958).

2) If *cys-c* and *cys-t* were *not* at separate loci along the linear axis of the chromosome, the two classes of convertants with nonparental combinations of markers might still occur in different frequencies, if these three conditions prevailed:

a) gene conversion and crossing over are separate events;

b) *cys-c* and *cys-t* are converted to *cys*+ with different frequencies;

c) the amount of crossing over in the adjacent marked region on the left of *cys* is different from that on the right.

Under these conditions the majority type would be that produced by the more frequent conversion accompanied by the more frequent crossover. In the present crosses the *cys-c* gene appears to be the more frequently converted to *cys*+ (from the numbers of convertants with parental combinations of markers) and the more frequent crossover is in the *cys-ylo* region (Table 3). This combination of more frequent events would yield a *cys*+ convertant with the left-hand marker from the *cys-c* parent and the right-hand marker from the *cys-t* parent. However, this is the class that is consistently *less* frequent in the data than the reverse combination. Therefore, this explanation is untenable for the present case.

It should be pointed out that the *cys-c* parent carries the *un* marker in all five crosses. Viability of strains carrying this marker could be involved in the different frequencies of the marker combinations accompanying conversion. This appears improbable, as the more frequent parental combination is that carrying *un*, while the more frequent nonparental combination is that carrying *un*+. However, a bias due to marker viability can only be ruled out decisively by the study of a cross segregating for the same markers in the opposite alignment.

The author is inclined to the view that conversion is a separate event from crossing over and that the mutant genes participating in conversion (in this case, at least) are arranged along the linear axis of the chromosome pair, possibly as illustrated in Figure 2. *Cys*+ progeny could result from either of two processes. Conversion of either *cys* mutant gene to *cys*+ could take place by a nonreciprocal copying error along the DNA side chain; this process would show a correlation with crossing over in the *un-cys-ylo* region if both processes were favored by the same special pairing conditions. Secondly, *cys*+ progeny could be produced by reciprocal crossing over along the chromosome backbone between the attachment points of the *cys* mutant side chains. Although reciprocal recombination between the *cys* mutants was not observed in the asci analyzed in the present study, such an event has been demonstrated in similar situations in yeast (ROMAN 1958) and Neurospora (CASE and GILES 1958).

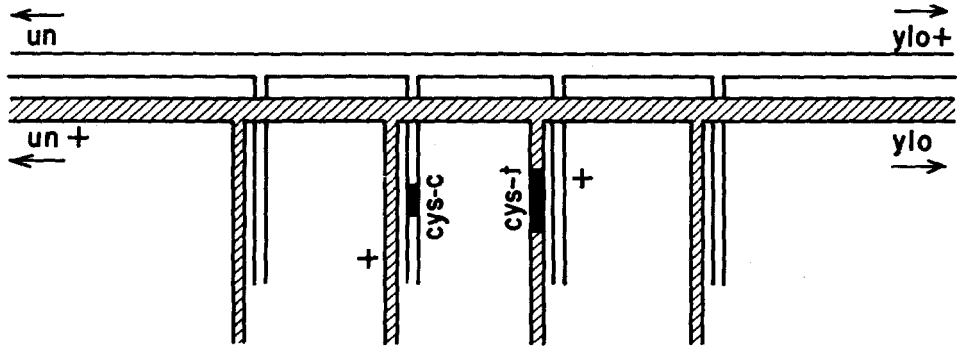

FIGURE 2.—See text for description.

SUMMARY

1. Crosses between two cysteine-requiring strains of Neurospora with mutant genes at the same locus or very closely linked loci yield 0.2 percent cysteine-independent progeny by a process other than crossing over; it is therefore termed "gene conversion."

2. Large changes in the recombination frequency for regions adjacent to the cysteine locus are not accompanied by extensive changes in the rate or pattern of gene conversion at this locus.

3. There is a high coincidence between gene conversion at the cysteine locus and recombination in the marked regions adjacent to this locus. The segment involved in such coincident recombination events appears to be shorter than the distance from the cysteine locus to the closest known marker on either side.

4. Five crosses between the two cysteine mutants have been studied in which linked markers were segregating on both sides. The relative frequencies of the different marker combinations accompanying gene conversion suggest that conversion and crossing over are separate events and that the two cysteine mutants are at separable loci along the chromosome.

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