

MEIOTIC GENE CONVERSION IN YEAST TETRADS AND THE THEORY OF RECOMBINATION¹

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CONVENTIONAL recombination theory has held that genetic recombination occurs after DNA replication, when each chromosome is composed of two chromatids; that crossing over involves a full-chromatid interaction between non-sister chromatids, and yields reciprocal products from breakage reunion events; and that either no crossing over occurs between sister chromatids, or if it occurs it is unrelated to homologous exchanges. Experience with this theory has shown that in a chromosome arm the occurrence of one exchange interferes with another exchange in an adjacent region. This has been manifested by detecting significantly fewer double crossovers than would be predicted on the assumption that individual exchanges occur independently of each other. Furthermore, among the multiple exchanges that do occur no evidence for chromatid interference has been found; two-, three-, and four-strand double exchanges are typically observed to occur in the expected 1:2:1 ratio (PERKINS 1962). This theory has had the consequence that multiply recombinated progeny derived from random sperm or random spores have been interpreted in terms of multiple exchanges between the genetic markers, even when the reciprocal products themselves have not been available for analysis.

However, recombination need not be a reciprocal process. The initial report by MITCHELL (1955) of nonreciprocal recombination (gene conversion) was followed by others (reviewed by WHITEHOUSE and HASTINGS 1965) from which the following has become clear. (1) Recombination within a cistron may be nonreciprocal for one or both of the input alleles. If the diploid is represented as $\frac{+ 1 + b}{a + 2 +}$ where 1 and 2 are two alleles of independent origin within a locus, and *a* and *b* are linked proximal and distal markers relative to the centromere, then 1 and 2 (or both) may segregate 3⁺:1⁻ or 1⁺:3⁻, while all other markers segregate in the normal 2⁺:2⁻ fashion. The 3⁺:1⁻ segregation results in a wild-type or prototrophic segregant; the 1⁺:3⁻ segregation results in a double mutant segregant. These results have been termed variously recombinants or convertants. (2) The flanking markers *a* and *b* may or may not be recombinated (+ + or *ab*) in the convertant strand or chromatid. (3) The proximal (1 +) or distal (+ 2) allele within a locus may consistently convert more frequently than the other allele. This has been termed polarization.

Recently, recombination models have been proposed (WHITEHOUSE 1963;

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HOLLIDAY 1964; WHITEHOUSE and HASTINGS 1965) which allow for nonreciprocal recombination. These postulate breakage of single nucleotide chains of DNA, followed by annealing between complementary chains from homologous chromatids. If such a "half-chromatid chiasma" spans a region of heterozygosity between the chromatids hybrid DNA (mispairing between bases) is formed. The mispaired bases can then be "repaired," presumably by enzymes. The repair process will yield a nonreciprocal result if an original mutant base pair is corrected to wild-type and on the homologous chromatid the original wild-type base pair is repaired to wild-type. The various theories differ in details as to the polarity of the interacting nucleotide chains and the requirements for additional DNA synthesis. EMERSON (1966) has attempted to quantify these theories for a single heterozygous site within a locus. The conventional situation above in which two different sites within a locus are heterozygous has proved much more refractory. None of the above models has completely accounted for the polarization effect. WHITEHOUSE (1966) suggests that the polarization could be a reflection of the underlying functional polarity of the cistron; perhaps the operator, which initiates m-RNA formation, also initiates nucleotide chain breakage in crossing over.

Critical evaluation of the reciprocal and nonreciprocal aspects of recombination would require a genetic system with the following attributes. (1) All the meiotic products must be available, and isolatable as tetrads. (2) Closely linked markers flanking the locus within which recombination is being studied must be present. The outside markers allow determination of chromatid involvement in crossing over. (3) A substantial sample size of recombinant tetrads must be available. In the study reported below, a system with many of the characteristics of the ideal one discussed above was designed in yeast, *Saccharomyces cerevisiae*, and 1109 recombinant tetrads were isolated from three different diploids. The resultant data are then analyzed, first by the classical theory of multiple exchanges, and then by the newer hypotheses cited above. These are then compared for their utility in understanding genetic recombination.

MATERIALS AND METHODS

The four histidine mutants used in these experiments originated as follows: hi_{1-1} from DR. C. RAUT via DR. D. HAWTHORNE, University of Washington (Seattle), in 1959; hi_{1-315} from DR. B. Z. DORFMAN, Yale University, in 1964 (his stock number E315); hi_{1-7} and hi_{1-204} from DR. R. MORTIMER, University of California (Berkeley), in 1962 and 1965 respectively (his stock numbers JB151 and JM204). Mutagens were ethyl methanesulfonate for mutant 315, ultraviolet light for 7 and 204, and probably for 1. The mutants were repetitively crossed to uniform backgrounds to extract derivatives with appropriate markers, and high sporulation and spore-survival. All crosses were made by mass mating the parental haploid cultures on agar solidified media containing yeast extract-peptone-dextrose (YEPD), and then replica-plating to synthetic complete plates deficient for threonine and arginine for zygote isolation. For details of media see ROMAN (1956), FOGEL and HURST (1963) and HURST and FOGEL (1964). Diploid cultures were sporulated on potassium acetate medium supplemented with 10 mg of adenine per liter. The zygotes synthesized by combining ascospore derivatives were as follows:

Z1958:

A6786C	<i>a</i>	+	+	<i>hi</i> ₁₋₇	+	<i>ar</i> ₆	<i>tr</i> ₂	<i>le</i> ₁	<i>ad</i> ₆	<i>gal</i> ₂	<i>ad</i> ₁
A6781A	α	<i>ur</i> ₃	<i>thr</i> ₃	+	<i>hi</i> ₁₋₁	+	+	+	+	+	<i>ad</i> ₁

Z2367:

A8425B	α	+	+	<i>hi</i> ₁₋₃₁₅	+	<i>ar</i> ₆	<i>tr</i> ₂	<i>le</i> ₁	+	+	<i>ad</i> ₁
A8067B	α	<i>ur</i> ₃	<i>thr</i> ₃	+	<i>hi</i> ₁₋₁	+	+	+	<i>ad</i> ₆	<i>gal</i> ₂	<i>ad</i> ₁

Z2433:

A8530B	α	<i>ur</i> ₃	+	<i>hi</i> ₁₋₃₁₅	+	<i>ar</i> ₆	<i>tr</i> ₂	<i>le</i> ₁	+	+	<i>ad</i> ₁
A8506C	α	+	<i>thr</i> ₃	+	<i>hi</i> ₁₋₂₀₄	+	+	+	<i>by</i> ₁	<i>gal</i> ₂	<i>ad</i> ₁

The brom-yellow (*by*) gene, discovered in this laboratory but previously unreported, is unlinked to any other marker in these diploids and shows no centromere linkage. The mutant accumulates or binds the acid form of brom-thymol blue from media alkaline to the indicator, causing white clones to become bright yellow, and red clones (*ad*₁ or *ad*₂ mutants) to become deep orange. The uracil-3 (*ur*), threonine-3 (*thr*), histidine-1 (*hi*), arginine-6 (*ar*), and tryptophan-2 (*tr*) markers are linked on linkage group V in the order given by MORTIMER and HAWTHORNE (1966). The centromere is located between *ur*₃ and *thr*₃, 5 centimorgans from *ur*₃ and 34 centimorgans from *thr*₃. The accumulated tetrad mapping data for the remaining markers on linkage group V are shown in Table 1, which summarizes our experience with 5460 tetrads (but all the markers were not segregating in each tetrad) and includes data on 3178 tetrads reported in HURST and FOGEL (1964). Markers other than those in Table 1 are located in three other linkage groups. Most of the additional asci were analyzed by a computer program developed by the Brooklyn College Computer Center. Table 1 shows the observed numbers of parental ditype, nonparental ditype, and tetratypes found among these asci; the exchange frequency is computed according to PERKINS (1949). Map distance is equal to one-half the exchange frequency. The order and indicated map distances in this linkage group are *thr*₃-2.4-*hi*₁-10.0-*ar*₆-20.8-*tr*₂. The diploid cultures were red, and required adenine and histidine. They were further purified by single-clone isolations from streak cultures on synthetic complete agar.

Asci containing prototrophic spores were identified, recovered, and analyzed by following technique. An inoculum of sporulated cells (asci) was removed from a sporulation plate and incubated in dialyzed snail enzyme (FOGEL and HURST 1963) for 10 minutes. After two washings in sterile distilled water, the asci were resuspended in methyl cellulose (1.5%) and distributed by a micropipette in droplets (0.5 mm diameter) on agar slabs of synthetic complete media lacking histidine, at a density of approximately 20 asci per droplet. The slabs were either prepared in the late afternoon and incubated overnight in moisture chambers at 23°C, or prepared and incubated directly at 28°C for 6 hours. The inocula were then examined for asci containing a single budded ascospore. All four-spored asci of this type (containing one budded and three

TABLE 1

Tetrad analysis for linkage in Group V

Marker interval	Parental ditype (PD)	Non-parental ditype (NPD)	Tetatype (T)	Exchange frequency* (T + 6NPD)/(Total asci)
<i>thr</i> ₃ - <i>hi</i> ₁	3566	0	181	.0483
<i>thr</i> ₃ - <i>ar</i> ₆	3241	3	1016	.2427
<i>thr</i> ₃ - <i>tr</i> ₂	1669	70	2083	.6566
<i>hi</i> ₁ - <i>ar</i> ₆	2987	2	731	.1997
<i>hi</i> ₁ - <i>tr</i> ₂	1635	50	1759	.5979
<i>ar</i> ₆ - <i>tr</i> ₂	2470	30	1478	.4168

* Map distance = 1/2 exchange frequency.

Interval lengths are: *thr*₃-2.4-*hi*₁-10.0-*ar*₆-20.8-*tr*₂.

normal ascospores) were dissected and then transferred onto YEPD agar slabs, incubated at 28°C for 48 hours, and the subsequent clones were analyzed by replica-planting for genotype and for their surviving histidine alleles by the method described by HURST and FOGEL (1964). Allele testing involves backcrossing each ascospore to tester strains of the input histidine alleles, and then scoring of the resultant zygotes for homo- or heteroallelic response. Asci containing two budded ascospores (with two exceptions) were not selected for dissection, since at that time such asci were considered to represent pre-existing prototrophs in the vegetative population prior to sporulation. The number of such asci was never estimated. Only asci with three or four surviving ascospores were selected for further analysis. In a small number of cases, the prototrophic spore had divided two to four times prior to isolation. These provided an opportunity to detect post-meiotic segregation among the first few vegetative division products of the prototroph. In six such cases where two to six products were examined no heterogeneity was observed. Sectorial clones from single ascospores, which might have reflected such heterogeneity, were absent. The distribution of HI prototrophy among asci containing four, three, and two surviving spores for two representative samples of Z1958 is shown in Table 2. Among 9,632 asci sampled, the frequency of HI prototroph-containing asci ranged from 1 per 270 among four-spored asci to 1 per 518 among asci containing only two spores. When compared to the expectations based on random ascospore death, these results are consistent with the assumption that spore death and HI prototroph formation are independent. The technique described above selects only for events terminating in prototroph formation, but does not identify other possible conversional events.

Table 3 summarizes all the asci isolated in these experiments. The 25 normal asci were isolated in the early stages of perfecting the technique. The false asci were asci in which nearly all the segregations were aberrant, and probably represent manipulative errors or clumping of spores. This category also includes a negligible fraction of asci in which fewer than three spores survived, and which therefore could not be uniquely classified. As the sporulated cultures aged, spore survival after dissection decreased appreciably, and the lowered yield of analyzable tetrads necessitated termination of the experiments. Two asci were isolated which contained two HI prototrophic spores, and 21 additional asci contained a single HI prototrophic spore, but the segregations were aberrant (not 2+ : 2-) for the flanking *thr*, *ar* and *tr* loci. These 23 asci were omitted from further analysis, since recombination was not easily scored in these cases. The frequency of aberrant segregation for these flanking markers, 1 to 2% per locus, is not higher than the aberrant segregation frequencies reported for a large number of yeast loci by ROMAN (1963) and thus does not lend support for associating conversion at the HI locus with aberrant segregation for flanking markers. The asci containing at least three viable spores, and in which all other markers segregated normally, were grouped into 48 classes defined by the markers flanking the *hi_i* locus and the distribution of surviving *hi_i* alleles in each ascus. Three-spored asci in which the dead fourth spore could be assigned without ambiguity were included in the appropriate class, on the assumption that the missing spore would not have resulted in an aberrant segregation for any markers, and further that a spore with a parental combination for outside markers almost invariably carries the histidine allele which came into the cross with that marker combination. These assumptions are rarely violated. Three-spored asci in which the dead fourth spore could not be clearly assigned, because the *hi_i* allele might have been either

TABLE 2

Distribution of histidine prototrophy among asci

Number of spores per ascus	Number of asci examined	Number of HI prototrophs	Fraction prototrophs	Expectations based on random death of ascospores
Four	2968	11	1/270	1/270
Three	3040	10	1/304	1/360
Two	3624	7	1/518	1/540
Totals	9632	28	1/345	

TABLE 3

Summary of asci isolated from Z1958, Z2367, and Z2433

Ascus type	Zygote			Total asci
	Z1958	Z2367	Z2433	
Normal, no prototroph in ascus	22	0	3	25
False asci	31	22	14	67
2 HI spores	1	0	1	2*
HI prototroph present, but aberrant segregation for <i>thr</i> , <i>ar</i> , or <i>tr</i>	13	6	2	21*
HI, normal, classified and analyzed in following tables	603	290	188	1081
HI, 3-spored, unclassified	17	4	7	28
Total asci isolated	687	322	215	1224

* A table describing these asci has been deposited in the GENETICS Editorial Office, and is available from the Editor.

input allele or double mutant, were grouped separately as unclassified. The unclassified and aberrant asci were not included in any subsequent analyses of the data.

The ordering of the mutants within the *hi*₁ cistron was achieved by plating asci of the various diploids on histidineless media (HURST and FOGEL 1964). Figure 1 shows the resultant map. The allele order from proximal to distal is indicated as 315-7-204-1. Some map expansion (HOLLIDAY 1964) at the proximal end of the locus is apparent. This order was independently verified by the marker combinations found in asci containing double mutant (reciprocal recombinant) spores for each diploid.

RESULTS

The three diploids in this experiment yielded 1109 four-spored prototroph-containing asci in which at least three spores survived to produce macroscopic clones. Of these, 1081 asci could be grouped into 48 classes, and 28 asci were unclassifiable since the fourth spore necessary for unequivocal assignment to classes was inviable. All the asci are shown in Table 4. The spore order given is one selected for convenience of presentation. In the tables to follow, all based on Table 4, the unclassified asci have been omitted.

The 48 ascus-classes were further grouped into three broad categories possessing the following diagnostic attributes. (1) *Reciprocal recombinants*: Asci containing a prototrophic spore, a double mutant spore, and one each of the parental input histidine alleles (Classes 1-11). (2) *Conversions of the proximal allele*: Asci containing a prototrophic spore, one proximal allele, and two distal alleles (Classes 12-37). These show aberrant segregation for the proximal allele. (3)

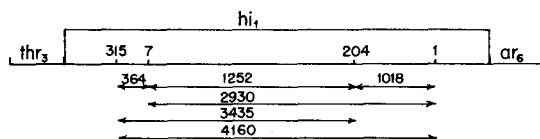


FIGURE 1.—Map of histidine alleles used in this study. The centromere is located on the *thr*₃ side of the locus; *ar*₆ is distal. The values are in prototrophs per million asci plated.

TABLE 4

Description of 620 HI prototroph-containing asci from diploid Z1958, 294 from Z2367, and 195 from Z2433, with respect to markers flanking the hi₁ locus, alleles surviving in ascus, and numbers of ascospores surviving in each tetrad. All crosses were of the general form shown below where 1 and 2 are the proximal and distal alleles respectively within the hi₁ locus



Ascus class	III prototrophic spore	Second spore	Third spore	Fourth spore	Asci analyzed						Total
					From Z1958		From Z2367		From Z2433		
					4's	3's	4's	3's	4's	3's	
1	-++---	+ 12 ++	-+2 ++	+1+---	53	5	13	2	2	0	75
2	-++---	+ 12 +-	-+2 ++	+1+--+	1	0	3	1	0	0	5
3	-++---	+ 12 ++	-+2 +-	+1+--+	1	0	0	1	0	0	2
4	-++---	- 12 ++	+2+ ++	+1+---	2	0	0	0	0	0	2
5	-++---	- 12 +-	+2+ ++	+1+--+	1	0	0	0	0	0	1
6	-++-+	+ 12 +-	-+2 ++	+1+---	5	0	0	0	0	1	6
7	-++-+	+ 12 ++	-+2 +-	+1+---	4	0	1	0	0	0	5
8	-++-+	+ 12 +-	-+2 +-	+1+--+	1	0	0	0	0	0	1
9	++++-	- 12 ++	-+2 -	+1++++	1	0	0	0	0	0	1
10	++++-	- 12 ++	-+2 ++	+1+---	2	0	0	0	0	0	2
11	++++-	- 12 +-	-+2 ++	+1+---	0	0	1	0	0	0	1
Total, asci containing reciprocal recombinants					71	5	18	4	2	1	101
12	++++-	-+2 ++	-+2 ++	+1+---	94	29	53	19	41	15	251
13	++++-	-+2 +-	-+2 -	+1+--+	40	10	14	5	14	6	89
14	++++-	-+2 ++	-+2 -	+1++++	14	6	5	5	5	1	36
15	++++-	-+2 -	-+2 +-	+1++++	1	1	0	0	0	0	2
16	++++-	-+2 ++	-+2 -	+1++++	1	0	0	0	0	0	1
17	++++-	-+2 ++	+2+ ++	-1+---	1	2	3	1	1	2	10
18	++++-	-+2 ++	+2+ +-	-1+--+	0	0	1	0	0	0	1
19	++++-	-+2 +-	+2+ ++	-1+--+	0	0	1	0	0	0	1
20	++++-	-+2 ++	-+2 +-	+1+---	38	8	14	6	9	4	79
21	++++-	-+2 ++	-+2 -	+1+--+	1	0	0	0	1	0	2
22	++++-	-+2 +-	-+2 -	+1+--+	1	0	0	0	0	1	2
23	+++++	-+2 ++	-+2 -	+1+---	32	15	19	3	7	6	82
24	+++++	-+2 +-	-+2 -	+1+--+	4	1	0	0	0	2	7
25	+++++	-+2 +-	-+2 -	+1+---	1	0	1	2	0	1	5
26	++++-	-+2 ++	-+2 -	+1+---	3	1	0	0	0	1	5
27	++++-	-+2 ++	-+2 -	+1+--+	1	2	0	0	1	0	4
28	-++---	-+2 ++	+2+ ++	+1+---	83	15	50	14	25	12	199
29	-++---	-+2 ++	+2+ +-	+1+--+	10	2	3	0	0	1	16
30	-++---	-+2 +-	+2+ ++	+1+--+	5	3	1	1	2	0	12
31	-++---	-+2 ++	+2+ -	+1++++	2	0	0	0	0	0	2
32	-++-+	-+2 +-	+2+ ++	+1+---	8	3	4	0	1	1	17
33	-++-+	-+2 ++	+2+ +-	+1+---	7	2	7	1	1	0	18
34	-++-+	-+2 -	+2+ ++	+1+---	1	1	0	0	0	0	2
35	-++-+	-+2 +-	+2+ -	+1+--+	1	1	0	0	0	0	2
36	-++-+	-+2 -	+2+ +-	+1+---	1	0	0	0	0	0	1
37	-++-+	-+2 +-	+2+ +-	+1+--+	0	0	1	0	0	0	1
Total, asci containing conversions of proximal allele					350	102	177	57	108	53	847

38	- + + + +	+ 1+ - - -	+ 1+ - - -	- + 2 + +	8	3	6	2	7	0	26
39	- + + + +	+ 1+ - - -	+ 1+ - + -	- + 2 + -	5	1	2	1	1	2	12
40	- + + + -	+ 1+ - - -	+ 1+ - + -	- + 2 + +	4	2	1	1	0	0	8
41	- + + - -	+ 1+ - - -	+ 1+ + + +	- + 2 + +	25	6	12	0	8	3	54
42	- + + - -	+ 1+ + - -	+ 1+ - + -	- + 2 + +	6	0	1	0	0	0	7
43	- + + - -	+ 1+ - + -	+ 1+ + + +	- + 2 + -	0	2	1	0	2	0	5
44	- + + - +	+ 1+ - - -	+ 1+ + + +	- + 2 + -	5	0	0	0	0	0	5
45	- + + - +	+ 1+ - - -	+ 1+ + - -	- + 2 + +	5	0	1	0	0	0	6
46	+ + + + +	+ 1+ - - -	- 1+ - - -	- + 2 + +	2	1	3	0	1	0	7
47	- + + + -	+ 1+ - - -	+ 1+ + + +	- + 2 - +	0	0	2	0	0	0	2
48	- + + + +	+ 1+ - - -	+ 1+ + + +	- + 2 - -	0	0	0	1	0	0	1
Total, asci containing conversions of distal allele					60	15	29	5	19	5	133
Total, classified					481	122	224	66	129	59	1081
..	- + + - -	+ 1+ - - -	- + 2 + +	missing	0	15	0	3	0	7	25
..	- + + - +	+ 1+ - - -	- + 2 + -	missing	0	2	0	0	0	0	2
..	- + + - +	+ 1+ - - -	- + 2 + +	missing	0	0	0	1	0	0	1
Total, unclassified					0	17	0	4	0	7	28
Grand Total					481	139	224	70	129	66	1109

Conversions of the distal allele: Asci containing a prototrophic spore, one distal allele, and two proximal alleles (Classes 38-48). These show aberrant segregation for the distal allele. The same general pattern was observed among the asci derived from each diploid. In all cases, asci of the second category, proximal allele convertants, represented the most frequently occurring single group and occurred at least six times more frequently than either reciprocal recombinants or distal allele convertants. Thus, a strong proximal polarization was observed. Although both reciprocal and nonreciprocal recombinants were observed among the asci from each diploid, asci in which nonreciprocal recombination had occurred (total 980) were about ten times more frequent than reciprocal recombinant asci (total 101). The proportion of asci containing double mutant spores varied from 12.6% in Z1958 to 7.6% in Z2367 to 1.6% in Z2433. The first two diploids involve crosses to a mutant at the extreme distal end of the histidine locus, while in the third cross the distal marker was located about one third of the distance from the distal end of the locus. The most frequent single class, 12, apparently due to proximal allele conversion, contained 251 asci, more than were derived from reciprocal recombinants and distal allele convertants together (total 234). Moreover, this class was not recombined for the outside *thr_s* and *ar_s* markers. The second most frequent class, 28, was recombined for these loci, but they did not contain a double mutant spore expected on the assumption that they had arisen by reciprocal recombination between the mutant sites.

ANALYSIS AND DISCUSSION

The Table 4 data may be analyzed in two major ways, depending upon which hypothesis of recombination is under examination. The first of these hypotheses

is the *multiple exchange hypothesis*. It assumes that essentially classical crossing over always occurs between homologous chromatids, and that all the conversional asci have arisen by an obligate exchange between the mutant sites. This often has the consequence of requiring many exchanges in a short region of the genome. In addition, for nonreciprocal asci in which 3:1 segregation has occurred, "conversion" of the appropriate allele must be postulated, without specifying at this time the mechanism leading to conversion. MURRAY (1963) analyzed a large body of random-spore data on these assumptions. The second hypothesis is stated in the contributions of WHITEHOUSE (1963), WHITEHOUSE and HASTINGS (1965) and HOLLIDAY (1964), in which recombination within a locus as the basis for gene conversion is envisaged as involving a DNA dissociation cycle, hybrid DNA formation, and repair of mispaired bases. In these models half-chromatid exchanges do not recombine outside markers, while full or whole chromatid exchanges do recombine outside markers. We propose to analyze the Table 4 data according to both hypotheses and then compare them.

Multiple exchange hypothesis: Interpretation in terms of classical exchanges begins with the assumption that the histidine prototrophs resulted from a single exchange between the two *hi*₁ alleles (region II in all following tables). For each tetrad, a chromatid diagram is constructed, and additional exchanges are postulated in the adjacent intervals to give the observed genotypes for each tetrad. Additional exchanges may be required in the region on the proximal side of the histidine locus with respect to the centromere (the *thr*₃ - *hi* interval, region I in all following tables) or in the intervals on the distal side of the histidine locus with respect to the centromere (the *hi* - *ar*₆ interval, region III, or the *ar*₆ - *tr*₂ interval, region IV in all following tables). Examples of such analysis are shown in Figure 2. In the nonreciprocal asci, it has been assumed that conversion involved the chromatid which participated in the exchange between the alleles.

Ascus Diagnosis	Class Number	Result	Multiple Exchange Hypothesis		Minimal Exchange Hypothesis	
			Chromatid Diag.	Scoring	Chromatid Diag.	Scoring
Reciprocal recomb.	1	- + + - -	+ 1 + - -	c.o. II	+ 1 + - -	c.o. II
		+ 1 2 + +	+ 1 ⊗ + - -		+ 1 ⊗ + - -	
		- + 2 + +	- + 2 + +		- + 2 + +	
		+ 1 + - -	- + 2 + +		- + 2 + +	
Proximal allele conversion	12	+ + + - -	+ 1 + - -	c.o. I, II	+ 1 + - -	no c.o.
		- + 2 + +	+ ⊕ + - -		+ ⊕ + - -	
		- + 2 + +	- + 2 + +		- + 2 + +	
		+ 1 + - -	- + 2 + +		- + 2 + +	
Distal allele conversion	38	- + + + +	+ 1 + - -	c.o. II, III	+ 1 + - -	no c.o.
		+ 1 + - -	+ 1 ⊗ + - -		+ 1 ⊗ + - -	
		+ 1 + - -	- + ⊕ + +		- + ⊕ + +	
		- + 2 + +	- + 2 + +		- + 2 + +	

FIGURE 2.—Interpretation of the prototrophic asci of Table 4 on the basis of multiple exchange and minimal exchange hypotheses. Circles denote the converted mutant site.

TABLE 5

Distribution of exchanges within the 1081 *HI* prototroph-containing asci of Table 4

Region	I		II		III		IV								
	+	<i>1</i>	+		<i>ar₆</i>		<i>tr₂</i>								
	<i>thr₃</i>	+	2	+		+	+								
		Number of asci containing exchanges in region(s):													
Ascus diagnosis	Zygote number	No. of asci	I II	I II IV	I II	I II IV	I II IV	II III	II III IV	II	II IV	II IV	I III	I II III IV	
Reciprocal recombinants															
	Z1958	76	4	1	0	0	0	0	0	58	11	1	1	0	
	Z2367	22	0	1	0	0	0	0	0	15	6	0	0	0	
	Z2433	3	0	0	0	0	0	0	0	2	1	0	0	0	
Conversion of proximal allele (1)															
	Z1958	452	123	96	3	1	0	4	3	98	40	0	67	17	
	Z2367	234	72	39	4	0	2	0	0	64	17	1	32	3	
	Z2433	161	56	33	3	1	0	0	0	37	6	0	19	6	
Conversion of distal allele (2)															
	Z1958	75	0	0	0	0	0	11	12	31	18	0	3	0	
	Z2367	34	0	0	0	0	0	9	7	12	3	0	3	0	
	Z2433	24	0	0	0	0	0	7	3	11	2	0	1	0	
Totals		1081	255	170	10	2	2	31	25	328	104	2	126	26	

The histidine prototrophs were assumed to arise from an exchange between the two *hi₁* sites (region II).

The resultant exchange distribution for each zygote and for each of the three major categories of prototrophic ascus is shown in Table 5. Only 328 asci were explicable merely by a single exchange between the *hi₁* alleles in region II; 753 required one or more additional exchanges in the adjacent regions. The observed exchanges in each interval, I, III, and IV, in Table 5 may now be compared to the expected number of exchanges in each interval based on the standard meiotic exchange frequency. The expected exchanges in each class are computed from the exchange frequencies for the appropriate interval from Table 1 times the number of asci in each major category. The comparisons of expected to observed exchanges are shown in Table 6. The same general pattern was observed for each diploid, and the results are summed in the last columns of Table 6. Among reciprocal recombinants, the exchange in region II which produced the prototroph did not interfere with exchange in the adjacent proximal interval I, but strong positive chiasma or chromosome interference was observed in the distal interval, region III. Only one exchange was observed, but 20 were expected. A less intense interference was observed in the further distal interval IV, where 26 exchanges were observed and 42 expected. Among proximal allele conversions, the exchange in region II enhanced exchange in the adjacent proximal interval about 15-fold; only 41 exchanges were expected, but 637 were observed. No interference was observed in the *hi-ar* interval, and 79% (280/353) of the expected

TABLE 6

The comparison of observed (O) with expected (E) *exchanges in each of the regions flanking the *hi*₁ locus within the 1081 *HI* prototroph-containing ascus of Table 4. The histidine prototrophs are assumed to arise from an exchange between the two *hi*₁ sites (region II)

Ascus diagnosis	Region				Zygote Z2367				Zygote Z2433				Totals								
	I		II		III		IV		I		III		IV		I		III		IV		
	<i>thr</i> ₃	+	1	+	<i>ar</i> ₆	2	+	<i>tr</i> ₂	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	
Reciprocal recombinants	O	76	6	1	18	22	1	0	7	3	0	0	1	101	7	1	26				
	E	..	4	15	32	..	1	4	9	..	0	1	1	..	5	20	42				
Conversions of proximal allele (1)	O	452	322	91	162	234	182	35	67	161	133	25	51	847	637	151	280				
	E	..	22	90	188	..	11	47	98	..	8	32	67	..	41	169	353				
Conversions of distal allele (2)	O	75	3	26	30	34	3	19	10	24	1	11	5	133	7	56	45				
	E	..	4	15	31	..	2	7	14	..	1	5	10	..	6	27	55				
Totals	O	603	331	118	210	290	186	54	84	188	134	36	57	1081	651	208	351				
	E	..	29	120	251	..	14	58	121	..	9	38	78	..	52	216	451				

* Expected exchanges = exchange frequency from Table 1 times the number of ascus.

exchanges were observed in region IV. Thus, proximal allele conversion was associated with negative interference in the proximal interval, and essentially no interference in the distal intervals. Among distal allele conversions, the exchange in region II produced no interference in region I, but enhanced exchanges twofold in region III, and occasioned no interference in region IV. Thus, among distal allele convertants, negative interference was observed in the adjacent distal interval, while no interference was detected in any other interval. When the pooled totals are examined, in the lower right of Table 6, the negative interference in region I is still clearly visible as a 12-fold increase in proximal exchanges over normal meiotic expectations. However, the summed region III exchanges are those expected from the standard meiotic map; the negative interference observed among distal allele convertants has been balanced by the positive interference among the reciprocal recombinants. No strong interference is observed in region IV since the observed exchanges are equal to 78% of the expected. MURRAY (1963), working with random spores in Neurospora, also observed increased exchanges in both regions flanking the *me₂* locus where conversion had taken place. In her data the strongest effect was a 67% exchange frequency in the distal interval. In the present study the observed exchange frequency in the proximal interval among histidine prototrophs was 60%.

The discussion has examined the exchange frequencies in regions flanking the histidine locus coincident with an exchange between the histidine alleles, and

TABLE 7

*Coincidence of double exchanges within the thr₃-tr₂ region: 3030 asci in five control experiments.**
No recombination occurred within the hi₁ cistron among these asci. Exchange frequency was calculated from tetrad data as in Table 1. All crosses were of the general type:

Region	I	II	III	IV
	+	1	+	<i>ar₆</i>
	<i>thr₃</i>	+	2	+

Zygote No.	Number of asci	Calculated exchange frequency			Observed numbers/expected numbers of tetrads		
		<i>thr-hi</i> I	<i>hi-ar</i> III	<i>ar-tr</i> IV	Pairs of regions		
					<i>thr-hi, hi-ar</i> (I, III)	<i>thr-hi, ar-tr</i> (I, IV)	<i>hi-ar, ar-tr</i> (III, IV)
Z1707	1020	.037	.222	.447	1/8.4 C=0.1	7/16.9 C=0.4	41/101.2 C=0.4
Z1958	605	.048	.185	.365	0/5.4 C=0.0	7/11.0 C=0.6	22/40.9 C=0.5
Z1959	357	.045	.154	.359	1/2.5 C=0.4	5/5.8 C=0.9	7/19.7 C=0.4
Z1960	589	.048	.200	.413	1/5.7 C=0.2	8/11.7 C=0.7	21/48.7 C=0.4
Z1989	459	.059	.176	.346	2/4.8 C=0.4	9/9.4 C=1.0	7/28.0 C=0.3
Total	3030	.046	.195	.398	5/27.2 C=0.2	36/55.5 C=0.6	98/235.2 C=0.4

* See Tables 8 and 12 for comparable analysis of HI prototroph containing asci.

has shown that among reciprocal recombinants positive interference was observed in the distal intervals, among proximal allele convertants negative interference was observed in the proximal interval, and among distal allele convertants negative interference was observed in the distal interval. For these intervals flanking the histidine locus, the coincident exchanges among prototrophic asci may be compared to nonconvertant control asci of similar genotype. Table 7 shows the coincident exchanges among 3030 control asci. The expected multiple exchanges are calculated from the observed exchange frequencies and the number of asci. The coincidence values (observed double exchanges/expected double exchanges) are 0.2 for I, III doubles, 0.6 for I, IV doubles, and 0.4 for III, IV doubles. PERKINS (1962) observed similar coincidence frequencies for a multiply marked chromosome in *Neurospora*. Table 8 shows the coincident exchange frequencies for the histidine prototrophic asci on the assumption of an exchange between the histidine alleles. Here too, the expected doubles were calculated from the observed exchange frequencies. Among reciprocal recombinants the sample sizes were too small for meaningful conclusions. Among proximal allele convertants, however, the coincidence is above one for the I, III interval, near one

TABLE 8

*Coincidence of additional double exchanges within the thr_3 - tr_2 region among the 1081 HI prototroph-containing asci of Table 4. The histidine prototrophs are assumed to arise from an exchange between the two hi_1 sites (region II). * The multiple exchanges are shown in Table 5; the exchange frequencies are derived from Table 6 totals*

Region	I		II		III		IV	
	$+$ thr_3	1 $+$	$+$ 2	$+$	ar_6 $+$	tr_2 $+$		
							Observed numbers/expected numbers of asci containing coincident exchanges in regions	
Ascus diagnosis	No. of asci	thr - hi I	hi - ar III	ar - tr IV	thr - hi , hi - ar (I, III)	thr - hi , ar - tr (I, IV)	hi - ar , ar - tr (III, IV)	
Reciprocal recombinants	101	.069	.010	.257	1/0 C=...	2/2 C=1.0	0/0 C=0.0	
Conversion of proximal allele (1)	847	.725	.178	.331	144/113 C=1.3	198/211 C=0.9	29/50 C=0.6	
Conversion of distal allele (2)	133	.053	.421	.338	7/3 C=2.3	0/2 C=0.0	22/19 C=1.2	
Totals	1081	.602	.192	.325	152/125 C=1.2	200/211 C=0.9	51/67 C=0.8	

* See Table 7 for control. See Table 12 for similar analysis on the minimum exchange hypothesis.

for the I, IV interval, and normal for the III, IV interval. Among distal allele convertants, the coincidence is above one for both the I, III and III, IV intervals. Thus, the multiple exchange hypothesis yields an excess over expected of double exchanges in the regions flanking the conversion locus.

Prototrophy at the heteroallelic histidine locus has been clearly associated above with exchanges in the immediate proximal and distal intervals flanking the locus. How often is the prototrophic chromatid involved in these adjacent exchanges? In normal asci any given chromatid may be expected to be involved in half the exchanges within any interval, while the sister chromatid should be involved in the remaining half of the exchanges. Random involvement of a chromatid with associated exchanges should thus yield a frequency of 0.5. Table 9 shows that this is not at all the case for the prototrophic chromatid. Among reciprocal recombinants, the expected value is observed in regions I and IV, and only one exchange was observed in region III, in which the prototrophic chromatid was not involved. Among proximal allele convertants, however, the prototrophic chromatid was involved in 91% of the proximal exchanges and 72% of the adjacent distal exchanges in region III. But it was only randomly involved in exchanges in region IV. Among distal allele convertants the prototrophic chromatid was involved in all the exchanges in regions I and III, and only randomly involved in region IV exchanges. Thus exchanges in the immediate proximal and distal intervals are intimately involved in the event or events which lead to the formation of a prototrophic chromosome.

In summary, a multiple exchange hypothesis which required that histidine prototrophs arose from a single conventional chromatid exchange between the two histidine alleles generated the following observations. (1) Among reciprocal recombinant asci, positive interference was seen in distal regions III and IV. (2) Among proximal allele convertants, a 15-fold excess of exchanges was found in the proximal interval, no interference was seen in regions III or IV, an excess of coincident double exchanges in the intervals flanking the histidine locus was required, and the prototrophic chromatid was nearly always involved in the proximal or distal exchanges in the immediately adjacent interval. (3) Among distal allele convertants, a twofold increase in exchanges in the distal interval was found, no interference in regions I or IV was observed, an excess of double exchanges in the intervals flanking the histidine locus was again required, and the prototrophic chromatid was always involved in the proximal or distal exchanges in the immediately adjacent intervals. Other multiple exchange hypotheses are also possible, for example one which allows more than one exchange between the mutant alleles. Such hypotheses would displace the exchanges postulated above to some other region, but would otherwise not change the conclusions in a major way.

Minimal Exchange hypothesis: Interpretation of the events leading to conversion in terms of minimal exchanges involves the assumption that exchanges are postulated only where strictly necessary. If the flanking markers are not recombined, then no exchange on the full chromatid level between homologous chromosomes is assumed, even though conversion has occurred within the hetero-

TABLE 9

*Frequency with which the H1 prototroph-bearing chromatid is involved in a proximal or distal exchange among the 1081 H1 prototroph containing asci, compared to total exchanges in the same intervals. The histidine prototrophs are assumed to arise from an exchange between the two h₁ sites (region II)**

Ascus diagnosis	Number of asci	Region				No proximal or distal exchange	Exchanges not involving prototrophic chromatid	Exchanges involving prototrophic chromatid in region(s)				Exchanges involving prototrophic chromatid					
		I	II	III	IV			I	II	III	IV	I	II	III	IV		
		+	1	+	ar ₆	tr ₂											
		thr ₃	+	2	+	+											
Reciprocal recombinants	101	75	10	4	0	12	0	0	0	0	0	0	0	4/7	0/1	12/26	0.5
Conversion of proximal allele (1)	847	199	30	391	5	36	94	83	9	0	0	0	0	577/637	108/151	128/280	0.46
Conversion of distal allele (2)	133	54	12	0	39	11	7	0	0	10	0	0	0	7/7	56/56	21/45	1.0
Totals	1081	328	52	395	44	59	101	83	9	10	83	9	10	589/651	164/208	161/351	0.90
														0.90	0.79	0.46	

* See Table 13 for similar analysis on the minimal exchange hypothesis.

TABLE 10

Distribution of exchanges within 1081 HI prototroph containing asci, Z1958, Z2367, and Z2433. The histidine prototrophs are assumed to arise according to the minimal exchange hypothesis.

Ascus diagnosis	Zygotite number	No. of asci	Region			No. of asci containing exchanges in region(s)																				
			Ascus rank			I II III IV																				
			0	1	2	3	I	II	III	IV	I	II	III	IV	I	II	III	IV								
			<i>thr</i> ₃	+	1	+	2	+	<i>ar</i> ₆	+	2	+	<i>tr</i> ₂	+												
Reciprocal recombinants	Z1958	76	0	58	15	3			0	58	0	0	4	0	0	11	0	0	1	1	0	0	1	0	0	1
	Z2367	22	0	15	6	1			0	15	0	0	0	0	0	6	0	0	1	0	0	0	0	0	0	0
	Z2433	3	0	2	1	0			0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Conversions of proximal allele (1)	Z1958	452	123	264	62	3			101	0	67	96	0	4	40	0	17	1	0	0	0	0	3	0	0	0
	Z2367	234	72	139	22	1			68	0	32	39	0	0	19	0	3	0	0	0	0	0	0	1	0	0
	Z2433	161	56	92	13	0			40	0	19	33	0	0	6	0	6	1	0	0	0	0	0	0	0	0
Conversions of distal allele (2)	Z1958	75	11	46	18	0			3	0	31	12	0	0	0	0	18	0	0	0	0	0	0	0	0	0
	Z2367	34	8	21	5	0			3	0	13	5	0	0	0	0	5	0	0	0	0	0	0	0	0	0
	Z2433	24	7	15	2	0			1	0	11	3	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Totals		1081	277	652	144	8			216	75	173	188	4	4	65	18	51	2	1	2	3	1	1	1	1	1

TABLE 11

The comparison of observed (O) with expected (E)* total exchanges in the regions flanking hi_1 locus within 1081 classified *HI* prototrophs containing *asci*, Z1958, Z2367, and Z2433. The histidine prototrophs are assumed to arise according to the minimal exchange hypothesis

Ascus diagnosis	Region				Zygotc Z1958				Zygotc Z2367				Zygotc Z2433				Totals									
	I		II		III		IV		I		III		IV		I		III		IV		I		III		IV	
	thr_s	+	1	+	ar_6	+	tr_2	+	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region	No. of asci	No. of exchanges in region
Reciprocal recombinants	O	76	6	1	18	..	4	15	32	22	1	0	7	3	0	0	1	101	7	1	26	E	..	4	15	32
Conversions of proximal allele (1)	O	452	148	91	162	..	22	90	188	234	88	35	67	161	46	25	51	847	282	151	280	E	..	22	90	188
Conversions of distal allele (2)	O	75	3	49	30	..	4	15	31	34	3	18	10	24	1	13	5	133	7	80	45	E	..	4	15	31
Totals	O	603	157	141	210	..	29	120	251	290	92	53	84	188	47	38	57	1081	296	232	351	E	..	29	120	251
	E	..	4	15	31	..	4	15	31	..	2	7	14	..	1	5	10	..	6	27	55		..	4	15	31
	E	..	22	90	188	..	11	47	98	..	8	32	67	..	9	38	78	..	52	216	451		..	14	58	121

* Expected exchanges = exchange frequency from Table 1 times the number of asci.

allelic interval. In the reciprocal recombinant asci, an exchange is again postulated between the alleles, but in the nonreciprocal asci, wherever an exchange is required between a flanking marker and a normally segregating (2:2) histidine allele, it is assumed to have occurred outside the histidine cistron. Examples of analysis on this basis are shown in Figure 2. It is seen that the most frequent item of Table 4, class 12, now has no exchanges, where under the multiple exchange hypothesis these required two-strand double exchanges in regions I and II. The Table 4 data analyzed by the assumptions described above result in the ascus ranks and exchange distribution shown in Table 10 for each zygote and major ascus category. This yields 277 tetrads in which no exchange at all was required to define the resultant ascospore genotypes. The exchanges within each interval may now be compared in Table 11 to those expected from the standard recombination frequencies. When Table 11 is compared to Table 6, which made the same comparisons for the multiple exchange hypothesis, it is seen that only two values have changed. The reciprocal recombinants are unchanged, since in both hypotheses under examination these are considered to represent single exchanges between the histidine alleles; positive chiasma interference is again observed in regions III and IV within this group. Among proximal allele convertants exchanges in region I are seven times more frequent than would be expected from the normal meiotic distance, and no interference is observed in regions III or IV. Among distal allele convertants, exchanges in region III are three times more frequent than expected, and no interference is observed in regions I or IV. In the pooled results, the negative interference in the proximal interval is still quite clear, but the distal effect is no longer apparent.

When the double-exchange coincidence values on the minimal switch hypothesis shown in Table 12 are compared to the control asci (Table 7) and to the coincidence values for the multiple exchange hypothesis (Table 8), it is seen that, with the exception of the III, IV double exchanges among distal allele convertants, the coincidence values in Table 12 are not significantly different from those observed among control asci. Thus, even though the exchange frequencies in region I among proximal allele convertants, and in region III among distal allele convertants, are much higher than normal, the I, III and I, IV double exchanges are not in excess when compared to normal asci. This stands in sharp contrast to Table 8, in which these same intervals showed coincidence values near or above one.

However, even assuming minimal exchanges, the prototrophic chromatid is still involved in nearly all the associated exchanges in the adjacent proximal and distal intervals, as seen in Table 13. The prototrophic chromatid was involved in 96% of the exchanges in the proximal region among proximal allele convertants, and to the same extent in the distal region among distal allele convertants. In all three major ascus categories, the prototrophic chromatid was only randomly involved in exchanges in region IV.

At best, the comparative analysis of chromatid interference on the multiple exchange and minimal exchange hypotheses is complex. In the multiple exchange analysis, numerous two-strand double exchanges were postulated (Table 5); 577

TABLE 12

Coincidence of double exchanges within the thr₃-tr₂ region among the 1081 HI prototroph containing asci of Table 4. The histidine prototrophs are assumed to arise according to the minimal exchange hypothesis

Region	I		II		III		IV	
	+	1	+	+	ar ₆	tr ₂		
	thr ₃	+	2	+	+	+		
							Observed numbers/expected numbers of tetrads	
							Pairs of regions	
Ascus diagnosis	Number of asci	Calculated exchange frequency						
		thr-hi I	hi-ar III	ar-tr IV	thr-hi, hi-ar (I, III)	thr-hi, ar-tr (I, IV)	hi-ar, ar-tr (III, IV)	
Reciprocal recombinants	101	.069	.010	.257	1/0 C=—	2/2 C=1.0	0/0 C=0.0	
Conversions of proximal allele (1)	847	.333	.178	.331	7/50 C=0.1	69/89 C=0.8	29/50 C=0.6	
Conversions of distal allele (2)	133	.053	.602	.338	0/4 C=0.0	0/2 C=0.0	25/27 C=0.9	
Total, minimal exchange hypothesis	1081	.274	.215	.325	8/64 C=0.1	71/92 C=0.8	54/76 C=0.7	
Total, multiple exchange hypothesis (Table 8)	1081	.602	.192	.325	152/125 C=1.2	200/211 C=0.9	51/67 C=0.8	
Total, control (Table 7)	3030	.046	.195	.398	5/27.2 C=0.2	36/55.5 C=0.6	98/235.2 C=0.4	

I, II doubles among the proximal allele convertants, and 53 II, III doubles among the distal allele convertants were required. Such two-strand double exchanges are ambiguously interpretable for an additional exchange or exchanges, because the nonprototrophic chromatid that has participated in the two-strand double is not distinctively marked for any subsequent exchange, compared to its sister chromatid which did not participate in the two-strand double. Thus, the pattern of chromatid involvement in exchanges could not be uniquely ascertained for the multiple exchange analysis. However, the excess of two-strand double exchanges described above strongly suggests that the classical 1:2:1 expectation for two-, three-, and four-strand double exchanges would not be found among these data, if analyzed on the multiple exchange hypothesis. No difficulty in ascertainment was present when the data were analyzed on the minimal exchange hypothesis. Table 14 compares the two-, three-, and four-strand double exchanges among the

TABLE 13

*Frequency with which the HI prototroph-bearing chromatid is involved in a proximal or distal exchange compared to total exchanges in same intervals among 1081 HI prototroph-containing asci. The histidine prototrophs are assumed to arise according to the minimal exchange hypothesis**

Ascus diagnosis	Number of asci	No proximal or distal exchange	Exchanges not involving prototrophic chromatid	Exchanges involving prototrophic chromatid in region(s)								Exchanges involving prototrophic chromatid								
				Region				Total exchanges in region				Total exchanges in region								
				I	II	III	IV	I	II	III	IV	I	II	III	IV					
				+	1	+	ar ₆	+	tr ₉											
				thr ₃	+	2	+		+											
Reciprocal recombinants	101	75	10		4	0	12	0	0	0	0	0	0	4/7	0/1	12/26				
Conversions of proximal allele (1)	847	251	140		229	94	83	5	36	9				270/282	108/151	128/280				
Conversions of distal allele (2)	133	26	13		7	66	10	0	0	11				7/7	77/80	21/45				
Totals	1081	352	163		240	160	105	5	36	20				281/296	185/232	161/351				
														0.95	0.8	0.46				

* See Table 9 for similar analysis on the multiple exchange hypothesis.

TABLE 14

Chromatid interference among 3030 control tetrads compared with 1081 tetrads in which HI prototrophy occurred

Region	I		II		III		IV					
	+	<i>1</i>	+		<i>ar₆</i>		<i>tr₂</i>					
	<i>thr₃</i>	+	2		+		+					
Double exchanges in regions												
Zygote number or source	I, III <i>thr-hi, hi-ar</i>			I, IV <i>thr-hi, ar-tr</i>			III, IV <i>hi-ar, ar-tr</i>			Pooled intervals		
	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str
Z1707	0	1	0	0	4	3	12	21	8	12	26	11
Z1958	0	0	0	1	4	2	7	10	5	8	14	7
Z1959	0	1	0	1	3	1	1	5	1	2	9	2
Z1960	0	0	1	2	6	0	5	11	5	7	17	6
Z1989	1	1	0	4	4	1	2	3	2	7	8	3
Total, control asci	1	3	1	8	21	7	27	50	21	36	74	29
Reciprocal recombinants	0	0	1	0	1	1	0	0	0	0	1	2
Conversion proximal allele (1)	2	5	0	19	34	12	6	14	9	27	53	21
Conversion distal allele (2)	0	0	0	0	0	0	6	14	5	6	14	5
Total, HI prototrophic asci	2	5	1	19	35	13	12	28	14	33	68	28
Combined Total	3	8	2	27	56	20	39	78	35	69	142	57

The number of tetrads containing 2-, 3-, and 4-strand pairs of exchanges is given for each set of intervals. The histidine prototrophs are analyzed according to the minimal exchange hypothesis.

prototrophic asci with double exchanges in the same intervals among 3030 control asci of Table 7. Among the 3030 normal asci, 139 double exchanges were scored; the distribution of 36:74:29 for two-, three-, and four-strand double exchanges was not significantly different from the 1:2:1 expectation assuming no chromatid interference. In the 1081 prototrophic asci, assuming minimal exchanges, 129 double exchanges were scored, almost the same number of double exchanges in a sample only one-third as large. The distribution of 33:68:28 was also not significantly different from 1:2:1. Thus, while the prototrophic chromatid was nearly always involved in an exchange in an adjacent interval, the four chromatids participated randomly in coincident exchanges in other intervals and no chromatid interference was observed. PERKINS (1962) reported a similar distribution of two-, three-, and four-strand double exchanges in *Neurospora*.

In summary the minimal exchange hypothesis, in which full chromatid exchanges were not postulated unless required by outside marker recombination, gave the following results. (1) Among reciprocal recombinants exchanged in region II, positive chiasma interference was observed in regions III and IV, and no interference in region I. (2) Among proximal allele convertants, with no full chromatid exchange in region II, a sevenfold increase in region I exchanges over

normal asci was observed, no interference in regions III or IV, coincidence values similar to normal asci, no chromatid interference, and the prototrophic chromatid was involved in nearly all the exchanges in the adjacent proximal and distal intervals. (3) Among distal allele convertants, with no full chromatid exchange in region II, we observed a threefold increase in region III exchanges over normal asci, no chiasma interference in regions I or IV, the III, IV coincident exchange value was the only one significantly different from normal asci, no chromatid interference, and the prototrophic chromatid was involved in nearly all the exchanges in the adjacent proximal and distal intervals.

Choice between multiple exchange or minimal exchange models: The two analytical methods used above result in similar statements with regard to the reciprocal recombinant asci, excess exchanges in the proximal interval among proximal allele convertants, excess exchanges in the distal interval among distal allele convertants, and the involvement of the prototrophic chromatid in exchanges in the intervals on either side of the histidine locus. The major differences lie in the chiasma and chromatid interference values between ascus categories and between hypotheses, and in the double-exchange coincidence frequencies. Most serious are the implications of Table 15, in which crossing over in the most distal interval (Region IV) is examined in the prototrophic and in the control asci. We observe that in control asci, in the absence of recombination in the *thr₃-ar₆* region the exchange frequency in the *ar₆-tr₂* interval was 42.6%. This value was 18.6% among asci showing recombination in the *thr₃-ar₆* interval, yielding a coincidence of 0.44. Among the 980 nonreciprocal (conversion) asci, the exchange frequencies in region IV among parental combinations were 40 and 45% for proximal and distal convertants, but only 24 and 27% for asci showing

TABLE 15

Crossing over in the distal ar₆-tr₂ interval among 1081 asci prototrophic at the hi₁ locus and among 3030 asci in which prototrophy did not occur at the histidine locus. All crosses were of the general type shown

Region	I	II	III	IV
	+	1	+	<i>ar₆</i>
	<i>thr₃</i>	+	2	+
				<i>tr₂</i>
Crossing over in the <i>ar₆-tr₂</i> interval (region IV) among				
Ascus category	Parental combinations + <i>ar</i> , <i>thr</i> +		Nonparental combinations <i>thr ar</i> , + +	
Reciprocal recombinants	1/4		25/97	
	0.25		0.26	
Conversions proximal allele	190/479		90/368	
	0.40		0.24	
Conversions distal allele	22/49		23/84	
	0.45		0.27	
Control (nonprototrophic) asci	984/2308		134/722	
	0.426		0.186	

nonparental combinations for *thr* and *ar*. The coincidence is 0.61. The difference in coincidence value from controls might be due to the presence of large numbers of region I crossovers and relatively fewer region III crossovers in the prototrophic asci compared to the controls. If these data are viewed under the multiple exchange hypothesis, a contradiction results: an exchange between the *hi*₁ alleles produces positive chiasma interference among reciprocal recombinants (26% exchange frequency), but the same exchange accompanied by proximal or distal allele conversion produces no interference in the same interval. Alternatively, a single exchange in the *thr-ar* interval (between the *hi* alleles) produces positive interference, but a two-strand double exchange (one between the *hi* alleles and one either proximal or distal) accompanied by conversion produces no interference. This contradiction is not present when these data are considered under the minimal exchange hypothesis. This observation, that conversion does not interfere with crossing over in a nearby region, was also clearly made by STADLER (1959) in a random spore study in *Neurospora*. The absence of positive chiasma interference among the nonreciprocal asci favors interpretation on the minimal exchange hypothesis.

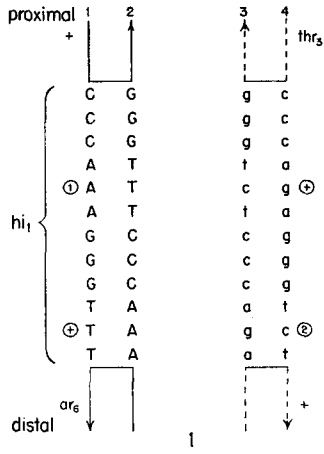
A similar discrepancy between the normal and prototrophic asci appears with regard to the double-exchange coincidence values. The multiple exchange hypothesis requires a negative interference effect in the nonreciprocal asci to the extent that, in the presence of an exchange between the histidine alleles, additional double exchanges occur in the intervals flanking the histidine locus (34 map units) with frequencies in excess of expectations based on the exchange frequencies in these same asci. The nonreciprocal asci on the minimal exchange hypothesis give double-exchange coincidence values similar to control asci, with one exception. The exception, an excess of III, IV doubles among distal allele convertants, will be discussed later.

Lastly, the multiple exchange hypothesis requires that 630 two-strand double exchanges be postulated among the nonreciprocal asci, which makes it unlikely that a classical pattern of two-, three-, and four-strand double exchanges would be observed. The minimal exchange hypothesis gives such a classical pattern, even though the prototrophic chromatid is involved in most exchanges in adjacent regions.

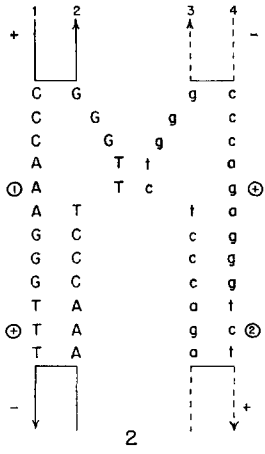
The argument above leads to the following conclusions: Either prototrophy within the histidine locus arises from a single unitary process involving full-chromatid exchange between the alleles, in which case reciprocal and nonreciprocal recombination possess widely different properties and require additional assumptions to satisfy the observations, or conceivably, the nonreciprocal prototrophs do not result from full chromatid exchanges between the alleles, while the reciprocal recombinants do. Thus, the question is: one recombination process or two? While the multiple exchange hypothesis may yet prove to be the more fruitful of the two, we prefer to seek hypotheses that do not assume full-chromatid exchange between the alleles as a prerequisite for prototrophy in nonreciprocal asci.

Hypothesis for gene conversion: A plausible hypothesis for gene conversion follows from the work of WHITEHOUSE (1963) and HOLLIDAY (1964), and critical suggestions by DR. M. MESELSON (personal communication). This hypothesis, shown in Figure 3, resembles HOLLIDAY's model and has the following assumptions. (1) A chromatid is composed of a single DNA double helix. After DNA replication and during effective pairing, a pair of cistrons, one in each homologue and differing at two sites within the cistron, are in very close apposition (Figure 3, 1). (2) At either heterozygous site or both, the base differences between the interacting chromatids creates stress. This leads to breakage of half-chromatids (strands) at the site of the stress. The polarity (3'-5' directionality) of the broken strands is the same (Figure 3, 2). (3) Each broken strand now anneals with the complementary strand of the homologous chromatid, generating a half-chromatid chiasma or exchange and mispairing a single base-pair in each chromatid (Figure 3, 3). (4) The mismatched bases will be "repaired" by an excision-and-replacement mechanism, either immediately or later. The repair presumably involves the inserted base, thus allowing repair of the original mutant to wild type or to the original mutant form, and repair of the original wild type to mutant or to wild type. If mutant is repaired to wild type, then a prototrophic chromatid may be generated. (5) The "half-chiasma" must be "terminalized" or resolved eventually, since the chromatids will separate at anaphase. Resolution may proceed in two ways, either by breakage and reunion of the strands originally involved (2 and 3) which does not recombine outside markers, or by breakage-reunion of the previously uninvolved strands (1 and 4) which will recombine the flanking markers. (6) No discontinuity is postulated within the DNA of the cistron, but discontinuity is present at both ends of the cistron.

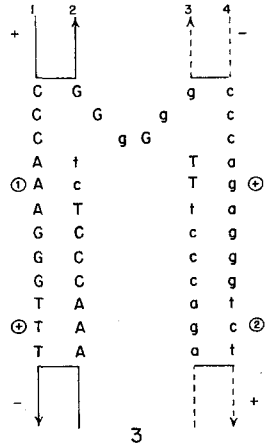
Half-chiasma resolution: Half-chromatid chiasmata, involving only one half of each homologous chromatid, do not themselves create chiasma interference in neighboring intervals. If resolution occurs by the 2-3 breakage (Figure 3, 4a), no interference would be expected, since a full-chromatid exchange is not present. If resolution occurs immediately by the 1-4 breakage, a full chiasma would be formed between the alleles, and classical chiasma interference should be expected. Suppose however that the half-chromatid chiasma began to terminalize by moving from the origination point to either end of the cistron. At any time during half-chiasma transit, resolution by 2-3 breakage may occur. If, during this passage, the second heterozygous site within the cistron is involved, then another pair of bases could become mismatched, affording the opportunity for repair at both mutant sites. But if resolution has not been attained when the half-chiasma reaches the discontinuity at the terminus of the cistron, then terminalization occurs by the 1-4 breakage (Figure 3, 4b), giving outside marker recombination that obligately associates the prototrophic chromatid with exchange in the adjacent interval. Terminalization can occur toward either end of the cistron, but appears to be more likely to occur in a direction to the nearest end, i.e., to the proximal end for a half-chiasma initiated at the proximal site, and to the distal end for a half-chiasma initiated at the distal site. Since terminalization to the end



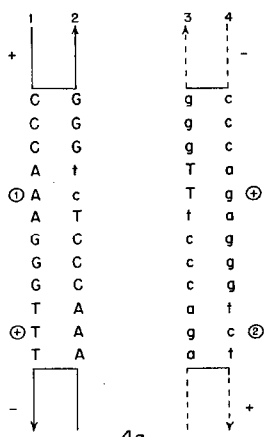
(pairing of homologous chromatids)



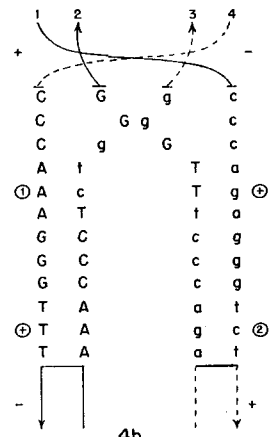
(breakage of non-complementary strands at proximal mutant site)



(formation of mispaired bases)



(strands 2 and 3 break and rejoin)



(strands 1 and 4 break and rejoin at cistron terminus)

of the cistron takes place in time, full chiasmata at the ends of the cistron may occur later in time than classical full chiasmata in neighboring intervals, and thus these do not interfere with each other.

Applying this hypothesis to the data reported earlier, the reciprocal recombinant asci were in the main half-chromatid chiasmata which were resolved by 1-4 breakage between the alleles. These engendered positive interference in the distal intervals. However, the absence of positive interference in region I among these asci suggests that at least some of them arose from the delayed half-chiasma resolution mechanism postulated above. The four asci of classes 9 to 11 (Table 4), all I, II multiple exchanges, could have been such tetrads. The proximal and distal convertant asci might also have contained some tetrads which had arisen by 1-4 breaks between the alleles, followed by repair to wild type. However, in view of the absence of interference in the adjacent intervals, these are considered to be low in frequency compared to terminalizations at the ends of the cistron, and thus all the exchanges in adjacent intervals among the allelic convertants were considered as exchanges at the ends of the cistron. The absence of chiasma interference among the III, IV double exchanges among distal allele convertants (Table 12) reflects the timing difference postulated above between classical exchanges and exchanges terminalized at the ends of a cistron. If the terminalization process by 2-3 breakage during transit to the ends of the cistron is a random process, then this hypothesis predicts that exchanges in adjacent intervals will be inversely proportional to the distance between a mutant site and the end of the cistron. A diploid composed of two mutant sites located in the middle of the cistron should show the lowest exchanges in the adjacent intervals. This view finds some support in the data of MURRAY (1963) in which exchange frequency in the proximal interval increased as the position of the proximal allele was displaced distally.

Polarity: While the above hypothesis formally accounts for most of the data, one observation, the consistent excess of proximal allele convertants over distal allele convertants, a proximal polarization, is not explained by the hypothesis. Proximal allele convertants were about six times more frequent than distal allele convertants (Table 4). No completely satisfactory explanation of this phenomenon is currently available. Speculatively, the polarization effect could result from preferential terminalization to one end of the cistron. WHITEHOUSE (1966) has suggested an operator model for recombination, in which the functional polarity of the cistron and the observed polarity in recombination are both regulated by the same operator segment of the cistron. Some characteristic of the operator segment could be the postulated discontinuity in the DNA. This might

FIGURE 3.—Diagram of the theory of gene conversion as applied to the *hi₁* locus in yeast. Solid lines and capital letters represent the DNA strand and bases of one chromatid, and broken lines and lower case letters represent those of the homologous chromatid. The polarity of the strands is indicated by the arrows, and the horizontal lines indicate the genetic discontinuity at the ends of the cistron. The circled symbols indicate the mutant sites within the cistron, and the proximal and distal markers are shown relative to the centromere position.

be supported by evidence showing that transcription and the directionality in recombination are initiated from the same cistron end for a diverse assembly of genes. For the *hi*₁ locus in yeast, only tenuous evidence exists (from derepressed mutants) that the proximal side of the cistron contains the operator site.

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SUMMARY

A total of 1109 asci containing histidine-prototrophs were scored from three different diploids heteroallelic for *hi*₁ in yeast. Each diploid carried three linked markers, two of which flanked *hi*₁. Of these, 1081 were classifiable into three broad categories: reciprocal recombinant asci (101), proximal allele convertant asci (847), and distal allele convertant asci (133). The 28 remaining could not be uniquely classified. The resultant data were then analyzed according to two major hypotheses. (1) The multiple exchange hypothesis, a unitary hypothesis in which every prototroph was considered to arise from a crossover between the alleles, and with additional exchanges postulated in the adjacent intervals to account for the resultant tetrad; and (2) The minimal exchange hypothesis, in which the reciprocal recombinant asci arose from exchanges between the alleles but the nonreciprocal prototrophic asci with parental outside marker combinations were assumed to have had no conventional crossovers in the interval under study, and if exchanges had to be postulated, these were assumed to occur in the intervals proximal and distal to the histidine locus, rather than between the alleles.—The reciprocal recombinant asci yielded identical results, regardless of hypothesis. These showed positive chiasma interference in the two distal intervals adjacent to *hi*₁. The nonreciprocal asci also gave similar qualitative results under the two hypotheses. The proximal allele convertant asci showed a 7- to 15-fold excess of exchanges in the proximal interval, and no chiasma interference in the two successive distal intervals. The distal allele convertant asci showed a two- to threefold excess of exchanges in the adjacent distal interval, and no chiasma interference in the other intervals. The prototrophic chromatid itself was involved in nearly all the exchanges in the adjacent proximal and distal intervals. The two analyses differed with respect to the double-exchange coincidence values and the relative frequency of two-, three-, and four-strand double exchanges. The multiple exchange hypothesis yielded coincidence values above one, and a large excess of two-strand double exchanges; the minimal exchange hypothesis resulted in values close to those observed in non-prototroph-containing control asci.—On the basis of these results, a recombination theory involving minimal exchanges is preferred. The one discussed differed only slightly from that of HOLLIDAY (1964).

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