Conversion-Associated Recombination in Yeast

(hybrids/meiosis/tetrads/marker loci/models)

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ABSTRACT   Gene conversion and conversion-associated reciprocal recombination have been studied in various *Saccharomyces cerevisiae* hybrids. In a sample of 11,023 unselected meiotic tetrads, 907 conversions were observed at the *arg4*, *thr3*, *his1*, and *SUP6* loci. Of these conversions, 445 (or 49.1%) were associated with reciprocal recombination of bracketing markers no more than 20-centimorgans apart. For conversions of two other loci, *his2* and *thr1*, for which the bracketing markers were more than 20-centimorgans apart, recombination frequencies were significantly greater than 50%. These findings are discussed in terms of current models of genetic recombination. It is suggested that all meiotic crossing-over is characterized by the recombination events that are associated with conversion.

In *Saccharomyces cerevisiae*, gene conversion for various heterozygous sites has been observed with frequencies from 0.1% to 20% (1, 2). For any given site, these aberrant meiotic segregations are detected as $3+:1a$ and $1+:3a$ ratios of meiotic products instead of the expected $2+:2a$. These two classes of conversional events are observed with about equal frequencies. When segregation of two heterozygous sites within a gene was analyzed in unselected tetrads it was found that, in addition to converting independently, the two alleles converted together (coconversion) in a symmetrical fashion (3–5). The frequency of coconversion increased at the expense of single-site conversions as the distance between the alleles was decreased (5). This finding implies that gene conversion is not restricted to a single nucleotide pair, as might be expected from a simple breakage event alone, but rather may involve a segment of DNA as long as 10^3 nucleotide pairs. Reciprocal recombination between two mutant sites within a gene was the least frequent of all intragenic events. However, high frequencies of reciprocal recombination between loci bracketing the converted locus have been observed (1, 6, 7). In this report, we summarize most of our data from unselected tetrads on coconversion and on conversion-associated reciprocal recombination.

MATERIALS AND METHODS

The procedures and media for hybridization, sporulation, ascus dissection, and scoring of genetic markers have been described (8, 9). Genetic marker designations are those proposed at the Osaka Yeast Genetics Conference (10). Three types of marker arrangements were synthesized for this study.

arg4 Diploids. *arg4* is located on chromosome VIII; the gene order and distances in map units (centimorgans, cM) are:

centromere *VIII* - 3.7 - *pet1* - 2.9 - *arg4* - 14.2 - *thr1* - 23.7- *CUP1* (5). The distal marker *thr1* was heterozygous in all diploids and *pet1* was heterozygous in most; where *pet1* was not present, the independently segregating centromere-linked genes *trp1* (chromosome IV) and *leu1* (chromosome VII) were used to assess second-division segregation for the proximal marker at the *arg4* locus. Six *arg4* alleles have been used. Four of these alleles, *4*, *1*, *2*, and *17* were described (5). Two additional alleles, *19* and *16*, have been located, with *19* between *4* and *1*, and *16* between *2* and *17*. If we modify the previous estimate (5) of the nucleotide-pair distance between these alleles according to the metric of Parker and Sherman (11) (1 x-ray map unit = 1 prototroph per 10^8 cells per roentgen = 129 nucleotide-pairs) the most proximal mutant *arg4-4* is about 700 nucleotide-pairs from the most distal one, *arg4-17*, and *arg4-1* is 340 nucleotide-pairs from *arg4-17*. The *arg4* genotype of each diploid is indicated in Table 1.

thr3 and his1 Diploids. Two diploids were studied, Z3735 and Z3910, in which the chromosome V genes *thr3*, *his1*, and *arg6* were heterozygous or heteroallelic. The Z3735 genotype was *thr3-1* + *his1-315* + *arg6*/+ *thr3-2* + *his1-1* + (two heterozygous sites within the *thr3* and *his1* genes, "heteroallelic," and one heterozygous site for *arg6*). The Z3910 genotype was *thr3 his1* +/+ + *arg6* (all heterozygous). The map order and distances are *centromere V* - 34 - *thr3* - 2.4 - *his1* - 10.0- *arg6* (7).

SUP6 Diploids. Diploids were also constructed in which the chromosome VI genes *his2*, *SUP6*, and *met10* were heterozygous. The map order and distances in the 2702 tetrads comprising this sample were *centromere VI* - 18.8 - *his2* - 9.0- *SUP6* - 2.6 - *met10*. These diploids were also heterozygous for the centromere-linked genes *trp1* and *leu1* and sometimes *ura3* (chromosome V); *thr1* and *CUP1* were heterozygous in 1992 of these tetrads.

Only complete tetrads were used in these studies. Ascospore viability for each diploid was greater than 90%. Where more than one heteroallele at a locus was segregating in a diploid, every ascospore clone of each unselected tetrad was tested (12) for the identity of the alleles at the locus with the modification that the selected diploids were exposed to nonlethal doses of ultraviolet light (240 ergs/mm²) or x-rays (2 kR) to enhance the ability to distinguish heteroallelic from homoallelic diploids. Every conversion tetrad was retested at least once. *SUP6* is an ochre suppressor that inserts tyrosine (13, 14) and is scored by maintaining the ochre mutants *lys1-1* and *ade2-1* homozygous in each diploid. The postmeiotic segregants for *SUP6* were detected upon replica-plating as sec-

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TABLE 1.   *Association of recombination with conversion in arg4*

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c        pet1       arg4        thr1
        /                    \
       4   19   1   2   16   17
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Diploid	arg4 genotype	Number of asci	Allele(s) converted	Interval	Number of conversions 3:1 plus 1:3	Number of conversions with recombination in interval
BZ34	4 +/+ 17	690	4	pet1-17	8	7
			17	4-thr1	42	19
			4-17	pet1-thr1	5	3
Z4049	4 +	517	4	c-2	1	0
Z3932	+ 2		2	4-thr1	18	10
			4-2	c-thr1	2	2
BZ28	+/17	313	17	pet1-thr1	22	10
Z3956	+ 17 / 2 17	243	2	pet1-thr1	22	13
Z3957						
Z3958						
BZ140	2 +/+ 17	544	2	pet1-17	1	1
			17	2-thr1	7	3
			2-17	pet1-thr1	28	14
X841	1 +/+ 2	367	1	pet1-2	3	1
			2	1-thr1	14	8
			1-2	pet1-thr1	19	8
X901	1 +/+ 2	116	1	c-2	2	2
			2	1-thr1	6	3
			1-2	c-thr1	5	4
X2961	19 + 17 / + 16 +	2566	19	pet1-16	19	12
			16	19-17	16	9
			17	16-thr1	41	21
			19-17	pet1-thr1	10	5
			19-16	pet1-17	2	2
			16-17	19-thr1	82	38
			19-16-17	pet1-thr1	42	21
X2988	1 2 + + / + + 16 17	1505	1	c-2	4	1
X2976			16	2-17	2	1
			17	16-thr1	15	3
			2-16	1-17	4	4
			1-2-16	c-17	2	1
			2-16-17	1-thr1	31	11
			1-2-16-17	c-thr1	74	31
Total		6861			549	268

tored ascospore clones. Each of these segregants was further analyzed by isolating and testing single clones from each side of the sector. The criterion for postmeiotic segregation required that all other segregating genes were identical in the two halves of the sector.

RESULTS

Conversion at the *arg4* locus

We have examined 6861 unselected tetrads from 13 different diploids in which one, two, three, or four mutant sites were segregating at the *arg4* locus. In all these asci, flanking markers on either side of the *arg4* locus (*centromere VIII, pet1* and *thr1*) were also scored, so that reciprocal recombination between these markers could be ascertained. Part of the sample (1743 tetrads) has been reported (5), but the numbers of conversion events reported here for these tetrads differs slightly from the previous report because of differences encountered in retesting. The 549 conversions observed among the 6861 asci are listed in Table 1 according to allele or alleles converted and associated recombination of bracketing markers. Thus, for the diploid BZ34, in which *arg4-4* and *arg4-17* were heterozygous, there were eight single-site conversions of *arg4-4*; seven of these were recombined for *pet1* and *arg4-17*, which bracket *arg4-4*. In addition, there were 42 single-site conversions of *arg4-17*; of which 19 were recombined for *arg4-4* and *thr1*. Finally, there were five double-site conversions in which both *arg4-4* and *arg4-17* were converted; of these, three were recombined for the bracketing markers *pet1* and *thr1*. The results from the other crosses are represented in Table 1 in a similar fashion. Coconversion of three and four mutant sites within a gene was observed in diploids X2961, X2988, and X2976. Among the 549 conversions, the numbers of 3+:1−

TABLE 2. *Frequency of conversion-associated recombination at arg4 relative to length of outside-marker interval*

Interval	Interval length (cM)	Number of conversions	Number of conversions with crossover	Fractions of conversions with crossover	X^2*
c-thr1	20.5	81	37	0.46	0.6
pet1-thr1	17.1	148	74	0.50	0.0
arg4-thr1	14.2	256	116	0.45	2.3
c-arg4	6.3	9	4	0.4	—
pet1-arg4	2.9	33	23	0.69	5.1†
arg4-a-arg4-b	<1	22	14	0.64	1.6
Total		549	268	0.49	0.31

* Chi-square test assumes that 50% of the conversions are associated with crossing-over.

† Significant at the 5% level ($P < 0.05$).

and $1+:3-$ segregations were about equal (287:262). The frequency of reciprocal recombination for bracketing markers among these 549 conversions was 48.8%, a value not significantly different from 50%. This observation was also independent of the number of sites converted: single-, double-, triple-, and quadruple-site conversions all show about 50% associated outside-marker recombination.

The data of Table 1 are rearranged in Table 2 according to the size of the genetic interval in which the reciprocal recombination occurred. For example, all the conversions for which centromere VIII - thr1 (c - thr1) defined the interval are combined in one line in Table 2. There were 81 such conversions; of these 37 (46%) were reciprocally recombined. Of particular interest are the 22 conversions represented in the last line of Table 2. These were observed in crosses in which three or four mutant sites within the arg4 locus were heterozygous; they represent conversion for the internal allele or alleles (for example arg4-16 in X2961) associated with $2+:2-$ segregations for the external alleles (arg4-19 and arg4-17 in X2961). In 14 of 22 conversions of this type, the bracketing alleles were reciprocally recombined. Thus, it is apparent that over the range from some hundreds of nucleotide-pairs to about 20 centimorgans (cM) the frequency of conversion-associated reciprocal recombination is, with one possible exception, essentially 50%. The absence of any correlation suggests that the conversion-associated recombination event occurs in the vicinity of the conversion itself, and, further, that additional, coincidental recombination within a 20-cM interval must be rare or absent. The results are consistent with the proposal that both conversion and recombination are outcomes of a single event.

Conversion at other loci

Results similar to those at the arg4 locus of chromosome VIII were found with heteroalleles at the thr3 and his1 loci of chromosome V, which was also marked by arg6 (Table 3). Coconversion was observed at each of the heteroallelic loci. One thr3-1 single-site conversion and four coconversions of the thr3 alleles are not included in Table 3, since the centromere V-thr3 interval was not marked and recombination associated with these conversions could not be assessed. The fraction of conversions with crossovers, $22/57 = 0.4$, is not significantly different from 50%.

In Table 4 are summarized the results from 26 related diploids in which the chromosome VI genes his2, SUP6, and met10 were heterozygous, and from which 2702 unselected tetrads were analyzed. In eight of these diploids, totaling 1992 tetrads, the chromosome-VIII genes thr1 and CUP1 were also heterozygous. The 50% relationship between conversion and crossing-over was again found for conversions at SUP6, where the flanking genes delimited an interval of 11.6 cM, but not for the his2 and thr1 genes, which were included within intervals of 27.8 and 44.5 cM, respectively, and for which the conversion-associated recombination frequencies were significantly higher than 50%. These higher frequencies could reflect the occurrence of additional independent crossovers somewhere within the larger intervals. In these same tetrads, 46 postmeiotic segregations for SUP6 were observed. 22 of these were of the $5+:3-$ type, and 24 were of the $3+:5-$ type. Of these 46 cases of postmeiotic segregations, 22 showed recombination for the flanking markers his2 and met10.

TABLE 3. *Conversion-associated recombination in chromosome V*

c   thr3 his1   arg6

Zygote	Number of asci	Allele(s) converted	Interval	Interval length (cM)	Number of conversions	Number of conversions with crossover	Fraction of conversions with crossover
Z3735	975	thr3-2	thr3-1-his1-315	2.6	7	3	0.4
		his1-315	thr3-2-his1-1	2.6	21	7	0.3
		his1-1	his1-315-arg6	7.4	1	0	0.0
		his1-315 and his1-1	thr3-2-arg6	10.0	13	5	0.4
Z3910	485	his1-16	thr3-arg6	8.8	15	7	0.5
Total	1460				57	22	0.4

TABLE 4.   *Conversion-associated recombination at his2, SUP6, and thr1*

Locus converted	Interval	Interval length (cM)	Number of conversions	Number of conversions with crossover	Fraction of conversions with crossover	Number of asci
SUP6	*his2-met10*	11.6	301	155	0.51	2702
his2	centromere-*SUP6*	27.8	381	241	0.63	2702
thr1	centromere-*CUP1*	44.5	144	109	0.76	1992

The data of Tables 2, 3, and 4 may be summarized for those intervals in which the bracketing markers are no more than 20-cM apart. In a total of 11,023 unselected tetrads, 445 of 907 conversions were associated with recombination, a frequency of 49.1%. As has been observed (3, 6, 7, 17), with rare exception, strands carrying the converted allele participated in the associated exchange.

DISCUSSION

The frequency of reciprocal recombination for genes bracketing a site at which gene conversion has occurred is about 50%. This relationship applies from intervals within the same gene, in which conversion has occurred, to intervals bracketed by genes about 20-cM apart. In addition, coconversion occurs for two, three, and four sites within a gene; exceptional (nonsymmetrical) conversion asci were very rare, and the 50% relationship was found regardless of the number of sites converted. Post-meiotic segregation has also been observed. These data may be compared with the predictions derivable from the molecular models of genetic recombination proposed by Whitehouse (15), Holliday (16, 17) and Stahl (18).

According to the Whitehouse (15) model, breaks occur in adjacent DNA double-helices, at a fixed point in single strands of opposite polarity. The single strands unwind and pair together to form hybrid DNA. After synthesis of DNA in the regions vacated by the broken strands, there is another cycle of strand separation and hybridization, degradation of surplus DNA, and finally correction of mismatched base pairs. If this process proceeds in both directions from the starting point, no recombination between bracketing markers is predicted; if this process proceeds in only one direction, then the bracketing markers would be reciprocally recombined. 50%-associated reciprocal recombination would occur only if the frequency with which this process operates in both directions is equal to the unidirectional frequency. Symmetrical coconversion could occur if two heterozygous sites are on the same side of the starting point and both are included in hybrid DNA. However, another constraint is required: the repair process must proceed by excision of a segment of a strand, which is then replaced by repair synthesis. Since it is unlikely that independent repair of hybrid DNA in two adjacent regions would always yield symmetrical coconversion as a result, none of the *arg4* alleles in this study could have been on opposite sides of a starting point in this model. Postmeiotic segregation is predicted if mismatched base pairs are not repaired before replication.

The recombination model of Holliday (16,17) starts with breaks in strands of the same polarity and proceeds in only one direction. Hybrid DNA is formed by pairing of the broken strands with the complementary strand of the homologous chromatid. This results in a "half-chromatid"; if this chiasma is resolved by breakage of strands 2 and 3 (the original broken strands) no recombination for bracketing markers is predicted. If the event is resolved by breakage and reunion of strands 1 and 4, then recombination for flanking genes should result. The 50%-associated reciprocal recombination obtains only if resolution by 2,3 breakage is equal to that by 1,4 breakage. Again, a constraint must be placed upon the repair mechanism involving repair of mis-matched base-pairs; not only must a segment of a single strand be excised and resynthesized to yield coconversion, but if hybrid DNA is present on both involved strands, two strands of opposite polarity must be partially excised and replaced. A repair that involved excision of two strands of the same polarity would generate normally segregating asci with two-strand double crossovers, a result seldom observed. Postmeiotic segregation is again predicted if mismatched base pairs have not been repaired.

Stahl (18) postulates that after meiotic pairing, an additional localized DNA synthesis occurs in homologous regions of a pair of chromatids. This generates "sex-circles", the arcs of which may then interact and recombine by breakage and reunion. Two exchange events are necessary; if these occur between the same two nonsister arcs, gene conversion will be observed for any heterozygous sites between the crossovers, and bracketing markers will remain parental in genotype. If all four arcs are involved in the two exchange events, flanking markers will be recombined. Excess DNA must be degraded in such a way as to preserve a pair of intact chromatids. Thus, 50% conversion-associated recombination would result if the strand involvement in the two crossovers is random. Coconversion would occur for all heterozygous sites between the crossovers; from our observation that mutant sites 700 or more nucleotide-pairs apart coconvert frequently, we conclude that the presumed sex circles must exceed that length. In this model, hybrid DNA is restricted to a short region in the vicinity of the exchange sites. Postmeiotic segregation would be expected only for unrepaired mutant sites in the exchange regions. We thus find that all three models, given some constraints on frequencies of strand breakage and reunion and on the mechanism of mismatched base-pair excision and replacement, allow for multisite conversion and the observed 50%-associated recombination.

Our major finding concerns the relationship between gene conversion and reciprocal recombination. Over a range of genetic map lengths, delimited by the flanking markers, from intragenic lengths to 20 cM, the frequency of conversion-associated reciprocal recombination is about 50%. This observation suggests that the crossover is causally associated with the conversion, and leads to the question: can all reciprocal recombination be accounted for by the events that lead to gene conversion?

This question is approached as follows. We may assume that the loci at which conversion has been studied represent a fair sample of the yeast genome. The average conversion fre-

quency at these loci, about 1%, should thus represent the fraction of the yeast genome that is involved in conversion in each meiosis (1, 5). The modal length of the converted segment has been estimated from multisite conversion data to be about 1000 nucleotide pairs. Conversion of 1% of the yeast genome (1.5×10^7 nucleotide-pairs) (19) would involve 1.5×10^5 nucleotide-pairs. This amount of converted DNA corresponds to 150 conversion events per meiosis. If half of the conversion events are associated with recombination, then 75 crossovers per meiosis are expected. If no other source of reciprocal recombination exists, this number of crossovers corresponds to a genetic length of the yeast genome equal to 3750 cM. This estimate compares favorably with the estimate of 3600 cM derived from genetic mapping results (20). Thus, we are encouraged in the view that crossing-over, associated with conversion, is sufficient to account for all meiotic recombination.

It is possible that the observation that 50% of conversions are associated with crossing-over, for intervals up to 20 map units, is only apparent. Because conversions not associated with crossing-over do not interfere with additional exchanges in the marked interval (6, 21) one should expect that as the marked interval is lengthened additional independent crossovers would occur. This would result in a linear increase in the frequency of crossing-over in the interval within which conversion has occurred, as the length of the interval is increased.

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