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Mitotic Gene Conversion Lengths, Coconversion Patterns, and the Incidence of Reciprocal Recombination in a *Saccharomyces cerevisiae* Plasmid System

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Plasmids capable of undergoing genetic exchange in mitotically dividing *Saccharomyces cerevisiae* cells were used to measure the length of gene conversion events, to determine patterns of coconversion when multiple markers were present, and to correlate the incidence of reciprocal recombination with the length of conversion tracts. To construct such plasmids, restriction site linkers were inserted both within the *HIS3* gene and in the flanking sequences, and two different *his3*$^-$ alleles were placed in a vector. Characterization of the genetic exchanges in these plasmids showed that most occur with the conversion of one *his3*$^-$ allele. Many of these events included coconversions in which more than one marker along the allelic sequence was replaced. The frequency of coconversion decreased with the distance between two markers such that markers further than 1 kilobase apart were infrequently coconverted. From these results the average length of conversion was determined to be approximately 0.5 kilobase. Examination of coconversions involving three or more markers revealed an almost obligatory, simultaneous coconversion pattern of all markers. Thus, when two markers which flank an intervening marker are converted, the intervening marker is 20 times more likely to be converted than to remain unchanged. The results of these studies also showed that the incidence of reciprocal recombination, which accompanies more than 20% of the conversion events, is more frequent when the conversion tract is longer than average.

Conversion is the nonreciprocal form of genetic exchange in which an allele, i.e., base sequence, found in one chromosome is replaced by the allele found in its homolog. Two models have been advanced to explain the molecular mechanism of conversion. The first mechanism (12, 19, 27) explains conversion as the repair of mismatched heteroduplex DNA formed between homologous DNA duplexes during genetic exchange. The other model (25) proposes that most conversion occurs by the synthesis of new DNA into a double-strand gap on a recipient DNA duplex, using the corresponding DNA sequence of the homolog as a template. In both models reciprocal recombination, which is known to accompany many conversion events, occurs during resolution of the joint structure formed by the exchanging duplexes during genetic exchange.

Fundamental to the understanding of the molecular basis of conversion is the knowledge of the average length of base pairs transferred and the pattern of resolution which occurs when multiple differences exist between the donor and recipient DNAs. Questions of conversion lengths and resolution patterns have been addressed by observing coconversions in which two or more markers are converted during a single exchange. For example, the average length of meiotic conversion events in *Saccharomyces cerevisiae* has been estimated to be less than the length of a gene on the basis of the observation that two mutations close together within a gene are more likely to coconvert than two markers at opposite ends of the same gene (6). In contrast, the length of mitotic conversion events in *S. cerevisiae* may be extremely large because coconversions of markers many centimorgans apart have been observed (8). For neither meiotic nor mitotic conversion has there been a systematic study of the functional relationship between length and the incidence of coconversion. Furthermore, questions concerning coconversion patterns have not been extensively explored partly because of the necessity of having three or more markers which span the conversion events. Evidence in *Ascobolus* species (9, 13) shows that the middle marker can remain unchanged while the flanking markers are coconverted. Although a direct observation like that made for *Ascobolus* species has not been made for *S. cerevisiae*, indirect evidence indicates that internal markers preferentially convert rather than remain unchanged (10, 21).

We undertook a systematic investigation of conversion lengths and coconversion patterns in mitotically dividing *S. cerevisiae* cells by using plasmids capable of undergoing genetic exchange (1). The plasmids contained heteroallelic copies of the yeast *HIS3* gene. By placing recognizable markers both within the gene copies themselves and within the flanking DNA, we were able to measure the length of coconversion events, classify their patterns, and explore their relationship with reciprocal recombination.

MATERIALS AND METHODS

Bacterial and yeast strains. *Escherichia coli* RR-1 *hisB recA* has been previously described (1). *S. cerevisiae* SSL204 (*MATα his3Δ200 trp1 leu2 ura3 ade2*) was constructed by mating a strain with the genotype *MATα rad52-1 trp1 leu2* to YNN217 (*MATa his3Δ200 ura3 ade2 lys2*; provided by Ron Davis, Stanford University [11]) and backcrossing spores containing appropriate markers twice with YNN217. The *his3Δ200* allele is a deletion which covers the entire coding sequence (11). The use of this allele decreases the possibility of genetic exchange between a plasmid and the chromosome.

Plasmid constructions. Standard cloning techniques were employed for plasmid constructions (18). Linkers were introduced into YIp1 (22) by partially digesting plasmid DNA

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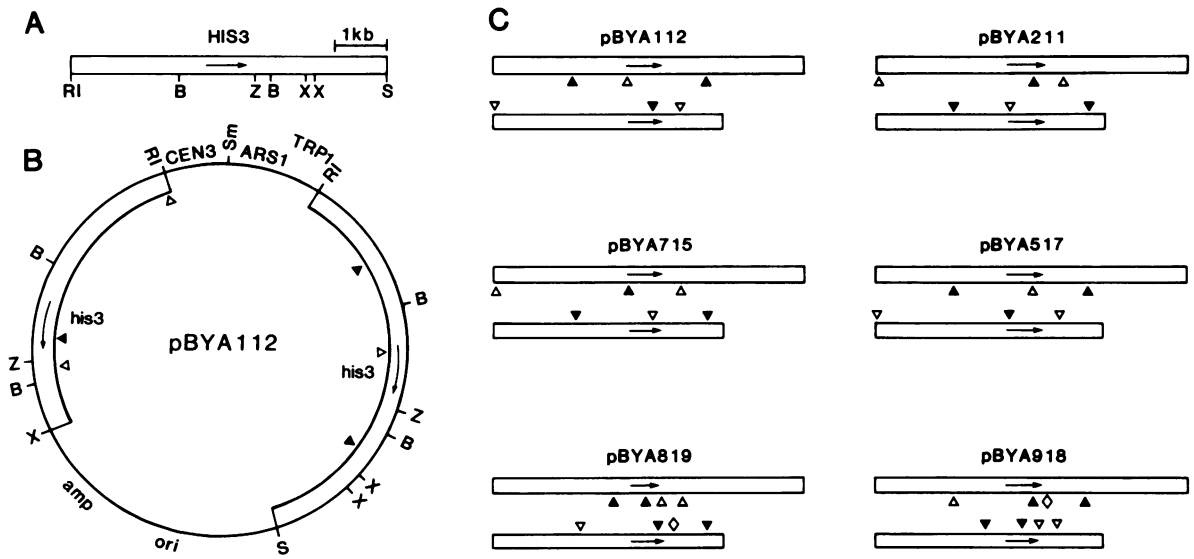

FIG. 1. Map of the yeast *HIS3* region and plasmids used in this study. (A) Restriction map of the 6.1-kb *HIS3* region from chromosome XV of *S. cerevisiae* (24). The arrow shows the direction and extent of the *HIS3* coding region. RI, *Eco*RI; B, *Bam*HI; Z, *Xho*I; X, *Xba*I; S, *Sal*I. (B) Diagram of one of the plasmids used in this study. The double line represents chromosome XV sequences. The single lines represent bacterial and yeast sequences needed for selection and propagation of the plasmid in the two organisms. The open triangles are *Cla*I linkers, and the closed triangles are *Sac*I linkers. Sm, *Sma*I. (C) Diagrams of the marker placements within the six plasmids used in this study. Only the two *HIS3*-containing segments from each plasmid are shown. The top line of each is the *Eco*RI-*Sal*I segment, while the bottom line is the *Eco*RI-*Xba*I segment. The scale and the placement of the coding region are as shown in panel A, and the open and closed triangles are defined in panel B. The diamonds are the new *Sma*I sites generated by the placement of *Sal*I-*Sma*I adapters into the unique *Xho*I site. With the *Eco*RI site as a reference position of 0.0 and reading along both allelic segments from left to right, the six markers in plasmids pBYA112, pBYA211, pBYA715, and pBYA517 are at positions 0.05, 1.6, 2.6, 3.1, 3.6, and 4.2 kb, respectively. The positions of the eight markers in plasmids pBYA819 and pBYA918 are 1.6, 2.2, 2.9, 3.1, 3.2, 3.3, 3.6, and 4.2 kb, respectively.

with *Hae*III, *Rsa*I, or *Alu*I, purifying full-length linear molecules, and ligating in the presence of phosphorylated 8-base-pair (bp) *Sac*I or *Cla*I linkers (New England BioLabs, Inc., Beverly, Mass.) to recircularize. In one case, *Sal*I-*Sma*I adapters (New England BioLabs) were used to join the unique *Xho*I site near the 3′ end of the *HIS3* coding region to create a 10-bp insertion and a new *Sma*I site. Ligation mixtures were transformed into *E. coli* RR-1 *hisB recA* and selected on plates containing ampicillin. Utilizing the known complementation of *E. coli hisB* mutations by the yeast *HIS3* gene (23), clones were screened for the presence of a mutated *HIS3* gene. Both *HIS3*$^+$ and *his3*$^-$ clones were saved. Extensive restriction analyses were carried out to locate the linker insertion, to ensure that no deletion of yeast sequences had occurred during the linearization process, and to confirm that only a single linker had been inserted. Next, using standard schemes, sequences with multiple insertions were constructed by combining individual insertion alleles.

To construct the plasmids containing heteroalleles diagrammed in Fig. 1, we introduced sequences containing multiple insertions into a vector as previously described (1) with two modifications. First, in previous constructions one allele was contained in a 1.75-kilobase (kb) *Bam*HI fragment, whereas in this study this allelic copy was a 6.1-kb *Eco*RI-*Sal*I fragment of yeast chromosomal DNA. Second, a 627-bp *Sau*3A fragment containing the *CEN3* sequence (5) was incorporated into position 1368 in the *Eco*RI fragment containing *ARS1* and *TRP1* (26) without disrupting these two markers. The *CEN* sequence was incorporated into our new constructions to reduce the plasmid copy number and to further decrease the possibility of intermolecular exchange either between two plasmids or between a plasmid and the chromosome. The low copy number and high proportion of cells with plasmid copies, which is also a property of the *CEN* sequence, permits the determination of exchange rates by fluctuation analysis (16, 17).

Analysis of exchange events. As previously described (1), heteroallelic plasmids were transformed into strain SSL204. Histidine auxotrophic clones were propagated in the presence of histidine and then plated to determine the frequency of histidine prototrophs. To analyze independent recombination events, we isolated plasmid DNA from a single prototrophic colony from each independent clone. The DNA was next transformed into *E. coli* RR-1 *hisB recA*. Control experiments performed in our previous study (1) and in this work show that the use of *E. coli* cloning after isolation of the recombined plasmid DNA from yeasts does not alter the frequency or outcome of the events. DNA prepared from a single, prototrophic bacterial colony was analyzed by restriction digestions. Appropriate digests led to the unambiguous identification of sites within the recombined plasmids. To determine whether reciprocal recombination had occurred, plasmid DNA was digested with *Sal*I and *Sma*I to permit analysis of the inversion of the plasmid sequences indicative of crossing over (1).

RESULTS

Measurement of exchange rates. Because deletion mutations behave like point mutations during recombination in yeasts (4, 15), we expected that our use of insertion mutations would not affect plasmid exchange events. To assess whether the insertions drastically altered plasmid exchange, we determined exchange rates by fluctuation analysis (16, 17) with the assumption of one plasmid copy per cell. The

TABLE 1. Analysis of plasmid recombination events which give rise to a wild-type *HIS3* allele

Plasmid	Rate (10^{-3} events/ generation)	No. of convertants[a]										No. of reciprocal recombinants[b]	Total[c]
		Asymmetric, continuous								Asymmetric, discontinuous	Symmetric		
		I	II	III	IV	V	I′	II′	III′				
pBYA112	3.5	31 (3)	17 (4)	5 (3)			14 (0)	5 (2)	0	13 (4)	14 (5)	0	99 (21)
pBYA211	3.1	18 (0)	13 (3)	4 (0)			39 (4)	11 (3)	0	7 (4)	5 (3)	3 (1)	100 (20)
pBYA715	1.2	23 (1)	25 (3)	9 (1)			15 (3)	12 (5)	0	8 (2)	4 (2)	4 (1)	100 (21)
pBYA517	1.1	13 (3)	12 (1)	0			44 (7)	10 (3)	0	10 (7)	6 (1)	5 (2)	100 (27)
pBYA819	0.42	5 (0)	8 (0)	18 (0)	10 (2)	7 (4)	21 (4)	5 (1)	5 (2)	6 (2)	10 (7)	5 (2)	100 (27)
pBYA918	0.31	1 (0)	8 (1)	5 (0)	3 (0)	2 (1)	37 (4)	13 (6)	8 (3)	10 (5)	12 (6)	1 (0)	100 (27)
Totals				237 (30)				239 (47)		54 (24)	51 (24)	18 (6)	599 (143)
% of total				39.5				40		9	8.5	3	100

[a] In these events, conversion occurs within the *HIS3* heteroalleles to generate one (or rarely two) wild-type allele(s). Some are associated with reciprocal recombination which alters the linkage of flanking markers (numbers in parentheses).

[b] In these events, no conversion occurs within the *HIS3* alleles themselves; rather, a crossover between the heteroalleles generates the wild-type allele. Most (12) do not include conversion of any marker, but some (6) include conversion of one or more flanking markers (numbers in parentheses).

[c] The number in parentheses is the total number of events which include reciprocal recombination.

results (Table 1) are similar to results reported previously by ourselves (1) and Keil and Roeder (14). As expected, plasmids pBYA819 and pBYA918 yielded lower rates because the heteroallelic markers in the *HIS3* copies are closer together in these plasmids than in the other four plasmids. The values and internal consistency of the rates indicate that the insertion mutations used and the placement of multiple inserts along the allelic sequences do not grossly affect the exchange rate.

Analysis of coconversion events. Previously (1), we found that most plasmid intramolecular exchange events which generate a *HIS3*$^+$ allele occurred by the mechanism of gene conversion. In this study we facilitated the analysis of conversion events by making *HIS3* alleles with insertions of restriction site linkers both in the gene and in the flanking homologous sequences. In all we used three pairs of plasmids (Fig. 1) and analyzed nearly 600 independent exchange events (Table 1). The events were assigned to the following categories.

(i) Continuous, asymmetric conversions. Most exchange events which generated a *HIS3*$^+$ allele did so by conversion, and often this was accompanied by coconversion of adjacent markers along the same duplex. These events, which constituted approximately 80% of the total (Table 1), are categorized as asymmetric and continuous in that change had taken place on only one of the two allelic copies and all markers within the tract of coconversion were simultaneously converted. We subdivided these events into classes, such as those shown in Fig. 2 and described in the legend, based on the number of converted markers. Assignments to these classes could be made even when reciprocal recombination accompanied the conversion.

(ii) Asymmetric, discontinuous conversions. Convertants in which multiple markers were involved along one of the original allelic segments, but in which converted markers flank on both sides internal markers which remained unchanged, constituted 9% of the events and are categorized as discontinuous, asymmetric events. (A detailed description of these events is given in Fig. A1 and Table A1 of the Appendix.)

(iii) Symmetric conversions. Convertants in which sequence information must have passed from both allelic segments and been received by both are classified as symmetric and constitute 8.5% of the events. Some, but not all, of these events might be interpreted as double-crossover products. (A detailed description of these events is given in Fig. A1 and Table A2 of the Appendix.)

(iv) Reciprocal recombinants. A few reciprocal recombinants were observed in which intragenic crossing over occurred between the heteroalleles to generate one wild-type allele and one double mutant, and the molecule had the inverted configuration indicative of reciprocal recombination. In this category no conversion occurred in the *HIS3* gene.

Measurement of coconversion lengths. The length of DNA participating in a conversion event can be estimated by examining the frequency of coconversions of known lengths. A plot of the percentage of coconversion events occurring along each of the two allelic sequences for each pair of plasmids versus the minimal length of DNA which spanned the coconversions (see the legend to Fig. 3) revealed a decrease in frequency versus distance. Less than 20% of the events extended beyond I kb. The average length was approximately 0.5 kb. The shortest conversion events were found in class 1 for plasmids pBYA819 and pBYA918. These events, which constituted only 10% of their category, extended less than the 300 bp from the *HIS3* marker in the donor allele to the first flanking marker. The longest events were class III′ in plasmids pBYA819 and pBYA918 which must extend more than 1.3 kb. We never observed conversion of a marker 2.5 kb from the marker within the *HIS3* gene in plasmids pBYA112, pBYA211, pBYA517, and pBYA715. Average conversion lengths did not vary greatly among the six plasmids or between the two allelic copies within a single plasmid. The similarity in the conversion lengths between the two alleles of one plasmid indicates that the constraint imposed by the limitation of homology extending on either side of the *HIS3* gene does not limit the extent of conversion in this system. For example, for pBYA112, pBYA211, pBYA517, and pBYA715, the most distant flanking marker in class III events and the flanking marker in class II′ events are 1.1 and 1.0 kb, respectively, from their corresponding *HIS3* marker, yet the first is only 200 bp from where homology ends, while the second is 1.6 kb from the end of homology. The incidence of these two classes follows the function of conversion length described by the other classes and therefore suggests that the proximity of the end of homology does not grossly interfere with conversion lengths in this system.

Patterns of coconversion. Because many coconversion

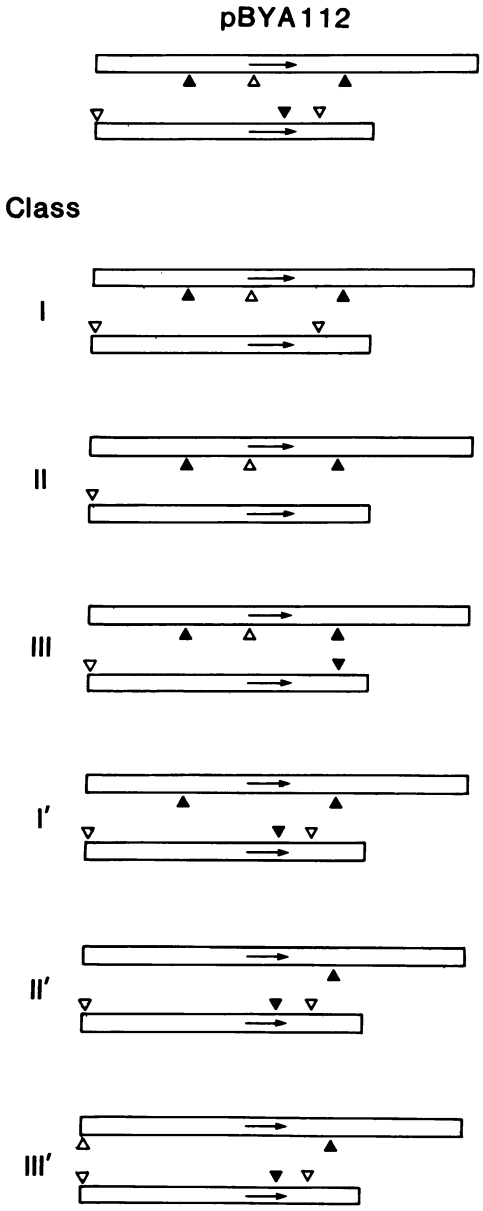

FIG. 2. Classes of continuous, asymmetric conversion events. The continuous, asymmetric conversion events were subdivided into classes according to the scheme shown here for plasmid pBYA112. For all plasmids (Fig. 1), numerals without primes refer to conversion of the right-hand (3') *HIS3* allele and coconversions extending to the right, whereas numerals with primes refer to conversion of the left-hand (5') allele and coconversions extending to the left. For plasmids pBYA819 and pBYA918 there are five classes of events with numbers lacking primes because of the addition of two markers between the 3' *HIS3* marker and the first flanking marker.

events included three or more markers, we were able to determine whether adjacent markers could be independently converted or whether all markers involved in a coconversion event were obligatorily converted in the direction of the allele providing the information. To examine this issue, we counted the number of events in which three or more markers were simultaneously coconverted and compared it with the number of discontinuous events in which internal markers failed to be converted. The results (Table 1) show 76 continuous events covering three or more markers (class III for pBYA112, pBYA211, pBYA715, and pBYA517; classes III, IV, V, and III' for pBYA819 and pBYA918). For the same intervals there were only four discontinuous events, which are included in Table 1 as part of the category of asymmetric, discontinuous conversions. (The description of these four events is given in Table A1 of the Appendix.) We conclude that internal markers are very likely to be converted if flanking markers are converted.

Although we observed a substantial number of asymmetric, discontinuous events, we limited our comparison to the four events described above. Except for these four events, all other asymmetric, discontinuous coconversions involved events in which markers on both sides of the *HIS3* gene were simultaneously converted along with the *his3*$^-$ allele on the recipient duplex, yet the linker marker within the *HIS3* gene on the donating duplex was not copied into the recipient (see Appendix). Considering the fact that we would not recover those in which the internal marker had been converted because they would have produced two identical but undetectable *his3*$^-$ alleles, we do not know the frequency of continuous events which encompass the same markers.

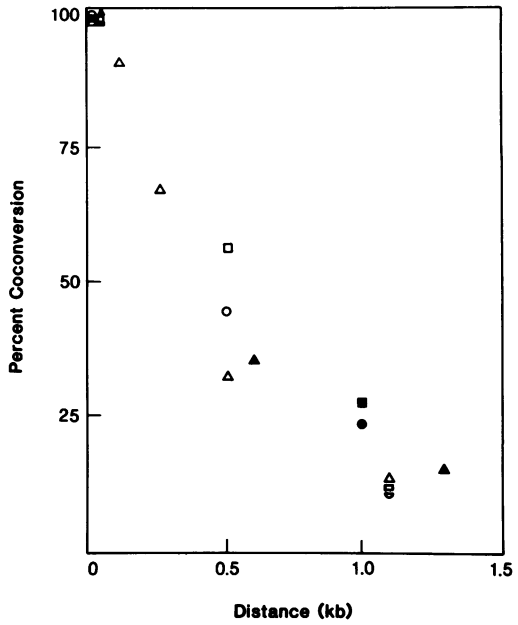

FIG. 3. Frequency of coconversion events as a function of distance. The data for the classes of continuous, asymmetric events listed in Table 1 are plotted as a function of distance. Each set of values represents the combined data for a plasmid pair, e.g., class III for plasmids pBYA112 and pBYA211. The distance is the shortest possible conversion length. For example, class III events for plasmid pairs pBYA112 and pBYA211 and pBYA715 and pBYA517 are plotted at a distance of 1.1 kb, because this is the distance between the 3' *HIS3* marker and the furthest 3' flanking marker. The frequencies have been plotted as a cumulative frequency of events which extend at least as far as the distance indicated along the respective allele. Thus, the values plotted at zero on the distance axis are 100% and represent all classes for the particular allelic sequence. Cumulative frequencies are used so data can be compared both between the two allelic copies within each plasmid and among all the allelic sequences of the three pairs of plasmids. Symbols: ○, ●, pBYA112 and pBYA211; □, ■, pBYA715 and pBYA517; △, ▲, pBYA819 and pBYA918. Closed and open figures represent classes with and without primes, respectively.

Consequently, we consider cases such as those for classes III, IV, V, and III′ from the six plasmids, in which recovery of both continuous and discontinuous examples is unbiased by selection, to be the more valid comparison.

Relationship to reciprocal recombination. In analyzing our results, we noticed a correlation between the incidence of reciprocal recombination and the length of conversion events. A total of 24% of exchange events included reciprocal recombination either in conjunction with the conversion giving rise to the *HIS3*$^+$ allele or as the cause of the wild-type allele. The numbers of reciprocal recombination events associated with the conversion categories are given in Table 1. In Fig. 4 the percentage of associated reciprocal recombination is plotted as a function of coconversion lengths for the classes of continuous, asymmetric convertants. The data indicate that reciprocal recombination is more likely to occur in conjunction with the more lengthy coconversion events than with simple class I and I′ conversions. Of 261 class I and I′ events, 29 (11%) occurred with reciprocal recombination, whereas 48 of 215 (22%) class II, III, IV, V, II′, and III′ events included reciprocal recombination. A chi-square analysis of these two ratios yields $P < 0.005$. Indeed, the chi-square value is actually biased in favor of finding the difference to be insignificant because many of the I′ events from plasmids pBYA112, pBYA211, pBYA715, and pBYA517 may be longer than the average of all events owing to the large distance to the first flanking marker.

In corroboration we note that two of our categories, the discontinuous, asymmetric events and the symmetric events, have an incidence of reciprocal recombination of nearly 50%. Events in these categories average 1.8 and 1.4 kb, respectively. Thus, their high incidence of reciprocal recombination is commensurate with the results of the continuous, asymmetric events. In this regard, the data of Roman (20) also portend the correlation of reciprocal recombination with longer, more complex events.

We were able to locate the positions of a few of the crossovers associated with the continuous, asymmetric convertants. Because the molecule is circular, the position of a reciprocal event with respect to the ends of the conversion tract cannot be determined if it occurs within the continuous tract. Only when a marker which has not been converted separates the crossover from the conversion tract can the position be designated. We found that of the 77 associated reciprocal events, 66 were within the conversion tract, while 11 were separated from the conversion tract by a marker which was not converted. Of those 11, 5 occurred with class II events, 1 with a class III event, 3 with class I′ events, and 2 with class II′ events. Seven took place with the marker separating the crossover being the heteroallelic marker in the *HIS3* gene, while the other four had a distal flanking marker separating the crossover position. Considering the small number of events, we tentatively conclude that associated reciprocal events are infrequently separated from the conversion tract by a marker which is not converted and that there is probably not a preferred position.

Polarity, marker preference, and other inequities. Plasmids pBYA112, pBYA211, pBYA517, and pBYA715 were designed with the intent of looking for inequities such as marker preference and polarity. Scrutiny of the data for the major category of continuous, asymmetric events given in Table 1 reveals that the most striking inequity in this plasmid system is a position preference in which the *his3*$^-$ allele in the *Eco*RI-*Xba*I fragment is twice as likely to be converted as the allele in the *Eco*RI-*Sal*I fragment (Fig. 1). Thus, for plasmids pBYA112, pBYA715, and pBYA819, classes I through V are found nearly twice as often as classes I′ through III′, whereas for plasmids pBYA211, pBYA517, and pBYA918, the primed classes are recovered twice as often as the classes without primes. (A chi-square analysis yields $P < 0.005$.) This position preference does not appear to create any further inequities such as major changes in average conversion length. Once the position preference is taken into consideration, other potential inequities can be examined. For example, there is no observable polarity in that for any pair of plasmids, e.g., pBYA112 and pBYA211, there are nearly equal numbers of convertants of the 5′ *HIS3* marker as of the 3′ marker. Similarly, there is no preference with regard to whether a *Sac*I or a *Cla*I linker occupies a particular site, e.g., compare pBYA112 with pBYA715. Finally, comparisons of the relative frequencies of class II and III events between pBYA112 and pBYA715 and between pBYA211 and pBYA517 reveals that the nature of the flanking marker does not influence the length of conversion. In these comparisons the first marker outside the *HIS3* gene in pBYA112 and pBYA211 is a linker insertion which is converted to the wild-type sequence in both class II and III events, whereas in pBYA715 and pBYA517 the same position is occupied by a wild-type sequence which is converted to the linker insertion in the respective class II and III events. Because the data indicate no gross differences in class frequencies between the comparable plasmids, we conclude that the presence of an insertion or deletion on the donating duplex has little effect on the conversion tract length.

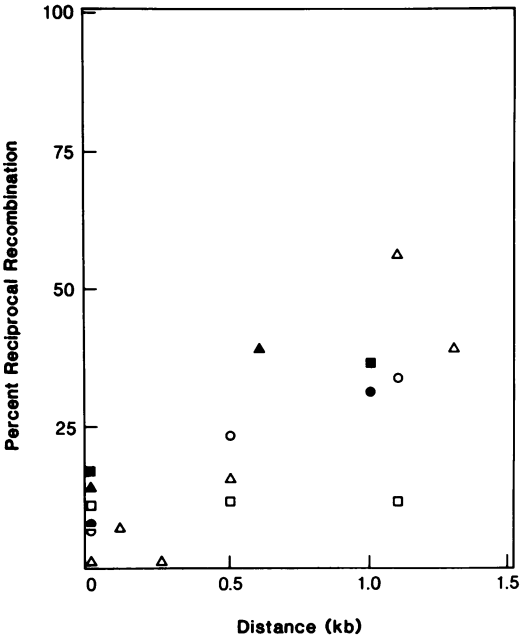

FIG. 4. Frequency of reciprocal recombination associated with conversion classes of given lengths. The percentage of reciprocal recombination which accompanied the coconversion classes within the asymmetric, continuous category are plotted as a function of the minimal distance of conversion as described in the legend to Fig. 3. Symbols are the same as those used in Fig. 3.

DISCUSSION

We manipulated the allelic copies of the DNA in our plasmid system to measure the length of conversion events

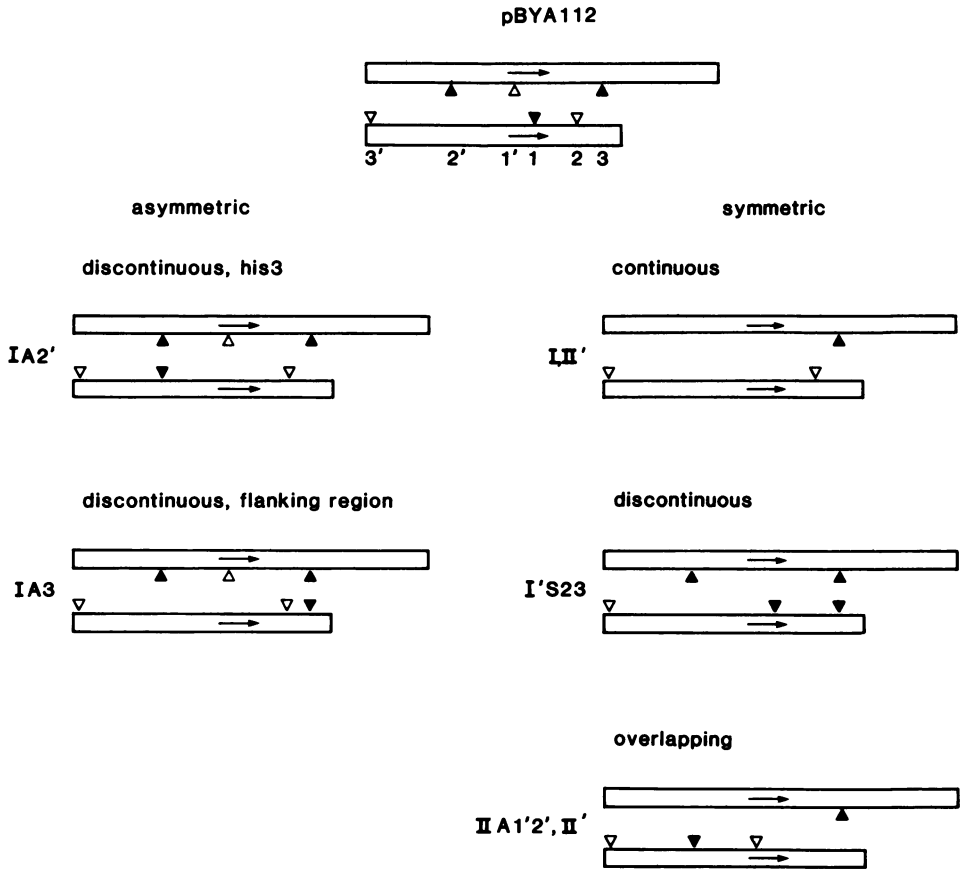

FIG. A1. Examples of asymmetric, discontinuous and symmetric convertants. Plasmid pBYA112 and five different classes of convertants are depicted. The molecules have been given a designation which follows from the classification scheme established in Fig. 2. For these designations all marker positions within the original plasmid have been numbered. Events are first designated by a roman numeral as shown in Fig. 2 to signify the conversion giving rise to the wild-type *HIS3* allele. Next follows a designation of the direction in which other markers are converted. An A, for asymmetric, is used to represent conversion of a marker along the same allelic segment, while an S, for symmetric, is used to represent conversion of a marker along the allelic segment opposite to that which is designated by the roman numeral. The arabic numerals following the letter designations identify which markers are involved. In giving a designation to the overlapping, symmetric events, the changes in both allelic segments are regarded to have occurred from the original molecule without any temporal order. Events which include reciprocal recombination are designated by an X (see Tables A1 and A2). For events which include a discontinuous pattern of coconversion, the conversion interval in which the reciprocal recombination occurs can be discerned. In those cases the X follows the designated interval. Designations within a set of parentheses precede an X when the position of the crossover within the included interval cannot be determined.

and observed patterns of coconversion. Using mitotically dividing cells, we showed that conversion events average a few hundred to one thousand base pairs in length. Two factors may make this an underestimate. First, we used the minimal possible tract length (see the legend to Fig. 3). Second, because we examined only histidine prototrophs, we selected for events which terminate between the heteroallelic markers. Thus, a longer average tract length might obtain if unselected recombination events had been examined. However, we do not believe our results are imposed by the physical constraints of our plasmids. Although these plasmids have limited homology between the allelic DNA sequences, the majority of events span no more than 20% of the available homology. Furthermore, the *HIS3* gene is located closer to one end of the homologous DNA than the other. If homology limits the length of conversion events, then conversion tract lengths should be shorter for the *HIS3* allele closer to the end of homology. Our results indicate that the conversion tracts radiating from the gene toward the ends of the homologous sequences do not differ between the two alleles.

The size range we observed contrasts with previous suggestions (3) that mitotic conversions might extend for many kilobases during chromosomal exchange. Although the difference between our conclusion and the previous suggestions may be due to the difference between intramolecular (plasmidic) recombination and intermolecular (chromosomal) exchange, we believe that the chromosomal measurements have yielded an overestimate because of the selection applied in the measurement of the tract lengths. Mitotic conversion lengths along chromosomes were not measured directly but were inferred from conventional genetic results. One such observation involves conversion between heteroalleles within a gene accompanied by reciprocal recombination in an adjacent region (2, 7). The observation is that the proximal heteroallele and the distal heteroallele, relative to the reciprocal recombination site, convert to wild type with equal frequency. This result

TABLE A1. Classification of asymmetric, discontinuous convertants[a]

Class	Classification with plasmid:					
	pBYA112	pBYA211	pBYA715	pBYA517	pBYA819	pBYA918
Discontinuous through *HIS3*	IA2′ IA2′ IA2′X IIA2′ IIA2′ IIIA2′ I′A2X II′A23 II′XA23 II′A3 II′A3 II′XA3 II′A23	IA2′X IIA2′ IIXA2′ I′A3 I′A3 I′A3X II′XA3	IA2′ IA2′ IIA2′ I′A2 I′A3 II′A2 II′XA3 II′XA23	IA2′ IXA2′ IIA2′ I′A3 I′XA3 I′XA23 II′XA23 II′XA23 I′A3 (X in 2′3′)	VXA2′ I′XA4 II′A4 II′A234	IVXA2′ I′A4 I′A5 I′A5 I′XA5 II′A5 II′XA25 III′A5 I′A5 (X beyond 3′)
Discontinuous in flanking region				IA3A2′X	IIA4 IIA5	I′A3′X

[a] The classification scheme is described in the legend to Fig. A1.

suggests that both heteroalleles are within a long stretch of heteroduplex DNA and that each converts independently. Although this interpretation likely is correct, we believe that the imposition of the requirement for reciprocal recombination may have obscured other events involving smaller conversion lengths. Our major category of smaller, continuous conversions does not have a high incidence of reciprocal recombination. However, we note that two of our minor categories, the asymmetric, discontinuous coconversions and the symmetric coconversions, resemble the events which support the conclusion that conversion tracts cover the length of a gene. In both minor categories the lengths of the conversion tracts are on average longer than the length of the gene and in both the incidence of reciprocal recombination approaches 50%.

Possibly, two pathways are operating on the plasmids. The first would yield relatively short, asymmetric, continuous conversion tracts with a low incidence of reciprocal recombination. In the second pathway, conversion lengths would be relatively long, heteroduplex more evident, and reciprocal recombination more frequent. Thus, the second pathway would account for our minor categories of asymmetric, discontinuous events and symmetric events as well as explain events which suggest that chromosomal mitotic conversion tracts are long. The only data which might support the prospect of a second pathway are that the incidence of events in the minor categories is slightly higher than what would be expected from their average size when compared with other events plotted in Fig. 3. However, their high incidence of reciprocal recombination cannot be used as justification for a second pathway because the values fit with the events plotted in Fig. 4. At this time we favor the concept of a single pathway which usually gives rise to small, continuous conversions but which infrequently yields the minor categories by virtue of the presence of small amounts of either asymmetric or symmetric heteroduplex. Possibly, only with longer conversion tracts does heteroduplex DNA, the presumed progenitor of discontinuous events, become more apparent.

Interestingly, our plasmid system has features of meiotic exchange. Even though the events occur in haploid cells during mitotic division, the length and asymmetry of plasmid events mimics meiotic exchange. Also, the exchange rate is more commensurate with meiotic than mitotic exchange, possibly resulting from the "forced pairing" present in the plasmids. Because the data are consistent with a single pathway and because the plasmid system has properties of meiotic exchange in a mitotically dividing cell, we believe a common pathway may exist in both meiotic and mitotic division. Differences in amounts of heteroduplex and position preference for initiation (polarity) may vary between the two division paths.

Our examination of coconversion spanning three or more markers bears on the mechanism of exchange. We found a disproportionate number of events in which all markers are converted in favor of the donor duplex. Clearly, independent correction within a conversion sequence is not the norm. Our result confirms in a direct manner the preponderance of conversions to restorations observed by Hastings and Savage (10, 21) for meiotic exchange in *S. cerevisiae*. Our result can be more easily accommodated in a model of recombination such as the double-strand-break repair model (25), in which an entire sequence is read from donor into recipient by a replicative process, than in models (12, 19, 27) which rely on mismatch correction within heteroduplex DNA to effect conversion. The caveat in using the double-strand-break repair model to explain our results is that asymmetric, discontinuous and symmetric events may originate from mismatch repair within heteroduplex. The double-strand-break repair model includes regions of heteroduplex flanking the double-strand gap which is formed during exchange (25), and this may account for the minor categories which we observe. We note that for the most part, the asymmetric, discontinuous and symmetric events which we observed (see Appendix) are not highly discontinuous, but are composits of short, continuous tracts. On the other hand, mismatch correction models can be invoked to explain our observations, if most of the repair tracts within heteroduplex are long, continuous, and concerted.

We found a higher incidence of reciprocal recombination in the coconversion classes, which reflect relatively long tract lengths, than in the single conversion classes. A comparison between this study and our previous study suggests a similar conclusion. Previously (1), we used plasmids which had only 1.75 kb of homologous DNA and found the incidence of reciprocal recombination to be 9% (8/88). In this study we used plasmids with 4.4 kb of homologous DNA and had an incidence of reciprocal recombination of 24%. (A

TABLE A2. Classification of symmetric convertants[a]

Class	Classification with plasmid:					
	pBYA112	pBYA211	pBYA715	pBYA517	pBYA819	pBYA918
Continuous	(I, I')X (I, I')X I, II' (I, II')X III, II' I'S2 I'S2 I'S2	III, II' (I'S2')X	(III, II')X (I'S2')X	I'S2'	IV, I' (IVS5)X (IVS5)X	
Discontinuous	IS2'X I'S23 IA3, I'		IA3, I'	IS2' I'A3S2'	I'S45X I'S45X I'S45X (I'S2')X5 II'(A5, S4)X	IS2' IS23(X in I'2') IA3'XS34 IIIA5, II' IIIS5 (V, I')XA3'
Overlapping	IIA1'2', II' IIIXA2 II'S1'2'	(IIS23)X (IIS23)X I'S1'	IA1'2'I'	IA1'2', I' (IIS23)X II'S2'	IV, I'S12 III'S3'	IIA5S345, II'S2' (VS24)X (VS4)X VA2'S45 VA2'S45 (III'S1')X

[a] The classification scheme is described in the legend to Fig. A1.

chi-square analysis yields $P < 0.01$ indicating a significant difference between our previous and current results.) The smaller homology present in our previous plasmid constructions may have been sufficient to limit the longer conversion events associated with reciprocal recombination. Two hypotheses can be advanced for the correlation between tract length and the incidence of reciprocal recombination. The incidence of reciprocal recombination could simply result from a probabilistic function in which it is equally likely, with each base pair of conversion, to incur reciprocal recombination. Hence, the cumulative probability would increase with distance. Alternatively, the correlation could result from a more fundamental mechanistic property in that isomerization, which might be required of a Holliday junction to yield a reciprocal recombinant, may not occur unless the conversion sequence reached a threshold length. Two observations suggest that heteroduplex plays a role in the threshold. First, the longer asymmetric, discontinuous and symmetric events, which we postulate to involve heteroduplex formation, have a high incidence of reciprocal recombination. Second, we noted that a small percentage of the crossovers associated with the asymmetric, continuous events were separated from the conversion tract by a marker which was not converted. The marker which separates a crossover from a conversion tract is also indicative of heteroduplex. Some perspective on the amount of heteroduplex which is involved in promoting reciprocal recombination comes from the measurement that the markers which separated crossovers from conversion tracts were approximately 0.5 kb distant from the tract. Whatever the mechanistic cause of the increased incidence of reciprocal recombination with the length of the conversion, our data provide the first indication of a physical constraint on the relationship between conversion and reciprocal recombination.

APPENDIX

Analysis of asymmetric, discontinuous and symmetric convertants. We classified the asymmetric, discontinuous and symmetric convertants according to a scheme which follows from the classification procedure depicted in Fig. 2 for the major category of continuous, asymmetric convertants. Figure A1 shows two classes of asymmetric, discontinuous convertants. In the major class, a conversion tract extends from a flanking marker through both markers within the *HIS3* gene, yet only the marker in the gene, which is distal to the converted flanking marker, is converted. This leaves an interrupted pattern of coconversion within the *HIS3* gene. Because coconversion of both markers in the gene would not result in a wild-type *HIS3* allele, we would not have recovered the continuous events for this interval. A minor class consists of an interrupted pattern in the flanking region. These rare examples have been discussed previously in regard to whether markers within a conversion tract are obligatorily converted or may remain unchanged. A list of all discontinuous, asymmetric events is given in Table A1.

The symmetric convertants have been divided among three groups. One class involves continuous events which involve conversion events in both allelic copies. Some, but not all, are similar to the example shown in Fig. A1 in that a simultaneous conversion of both mutations within the gene has occurred. A second class involves a discontinuous pattern of symmetric conversions. The third class has been termed overlapping in that one or more markers appear to have been reciprocally exchanged between the allelic segments. Although they can be interpreted as products of double crossovers, many are associated with molecules which exhibit the sequence organization indicative of a single reciprocal recombination event. A list of all symmetric convertants is given in Table A2.

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LITERATURE CITED

1. **Embretson, J. E., and D. M. Livingston.** 1984. A plasmid model to study genetic recombination. Gene **29**:293–302.

2. **Esposito, M. S.** 1978. Evidence that spontaneous mitotic recombination occurs at the two-strand stage. Proc. Natl. Acad. Sci. USA **75**:4436–4440.
3. **Esposito, M. E., and J. S. Wagstaff.** 1982. Mechanisms of mitotic recombination, p. 341–370. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Fink, G. R., and C. Styles.** 1974. Gene conversion of deletions in the *HIS4* region of yeast. Genetics **77**:231–244.
5. **Fitzgerald-Hayes, M., L. Clarke, and J. Carbon.** 1982. Nucleotide sequence comparisons and functional analysis of yeast centromere DNAs. Cell **29**:235–244.
6. **Fogel, S., R. K. Mortimer, and K. Lusnak.** 1982. Mechanisms of meiotic gene conversion, or wanderings on a foreign strand, p. 289–340. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Golin, J. E., and M. S. Esposito.** 1981. Mitotic recombination: mismatch correction and replicational resolution of Holliday structures formed at the two strand stage in *Saccharomyces*. Mol. Gen. Genet. **183**:252–263.
8. **Golin, J. E., and M. S. Esposito.** 1984. Coincident gene conversion during mitosis in *Saccharomyces*. Genetics **107**:355–365.
9. **Hastings, P. J., A. Kalogeropoulos, and J. L. Rossignol.** 1980. Restoration to the parental genotype of mismatches formed in recombinant DNA heteroduplex. Curr. Genet. **2**:169–174.
10. **Hastings, P. J., and E. A. Savage.** 1984. Further evidence of a disparity between conversion and restoration in the *his1* locus of *Saccharomyces cerevisiae*. Curr. Genet. **8**:23–28.
11. **Hieter, P., C. Mann, M. Snyder, and R. W. Davis.** 1985. Mitotic stability of yeast chromosomes: a colony color assay that measures nondisjunction and chromosome loss. Cell **40**: 381–392.
12. **Holliday, R.** 1964. A mechanism for gene conversion in fungi. Genet. Res. **5**:282–304.
13. **Kalogeropoulos, A., and J. L. Rossignol.** 1980. Evidence for independent mismatch corrections along the same hybrid DNA tract during meiotic recombination in *Ascobolus*. Heredity **45**:263–270.
14. **Keil, R. L., and G. S. Roeder.** 1984. *Cis*-acting recombination-stimulating activity in a fragment of the ribosomal DNA of *S. cerevisiae*. Cell **39**:377–386.
15. **Lawrence, C. W., F. Sherman, M. Jackson, and R. A. Gilmore.** 1975. Mapping and gene conversion studies with the structural gene for iso-1-cytochrome *c* in yeast. Genetics **81**:615–629.
16. **Lea, D. E., and C. A. Coulson.** 1949. The distribution of the numbers of mutants in bacterial populations. J. Genet. **49**:264–285.
17. **Luria, S. E., and M. Delbruck.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**:491–511.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Meselson, M. S., and C. M. Radding.** 1975. A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **72**:358–361.
20. **Roman, H.** 1980. Recombination in diploid vegetative cells of *Saccharomyces cerevisiae*. Carlsberg Res. Commun. **45**: 211–245.
21. **Savage, E. A., and P. J. Hastings.** 1981. Marker effects and the nature of the recombination event at the *his1* locus of *Saccharomyces cerevisiae*. Curr. Genet. **3**:37–47.
22. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosome segments with altered DNA sequences constructed *in vitro*. Proc. Natl. Acad. Sci. USA **76**:4951–4955.
23. **Struhl, K., and R. W. Davis.** 1977. Production of a functional eukaryotic enzyme in *Escherichia coli*: cloning and expression of the yeast structural gene for imidazole-glycerolphosphate dehydratase (*his3*). Proc. Natl. Acad. Sci. USA **74**:5255–5259.
24. **Struhl, K., and R. W. Davis.** 1980. A physical, genetic and transcriptional map of the cloned *his3* gene region of *Saccharomyces cerevisiae*. J. Mol. Biol. **136**:309–322.
25. **Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl.** 1983. The double-strand-break repair model for recombination. Cell **33**:25–35.
26. **Tschumper, G., and J. Carbon.** 1980. Sequence of a yeast DNA fragment containing a chromosomal replicator and the *TRP1* gene. Gene **10**:157–166.
27. **Whitehouse, H.** 1963. A theory of crossing-over by means of hybrid deoxyribonucleic acid. Nature (London) **199**:1034–1040.