FREQUENCY AND DIRECTIONALITY OF GENE CONVERSION EVENTS INVOLVING THE *CYC7-H3* MUTATION IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

The *CYC7-H3* mutation is a 5-kb deletion that causes overproduction of iso-2 cytochrome *c*. Unlike most mutations in yeast, the *CYC7-H3* mutation is preferentially lost when it is involved in a gene conversion event. We have shown that cloned copies of *CYC7-H3* DNA that are inserted into the yeast genome are associated with a high frequency of recombination and aberrant segregation events. Since parity in conversion frequency was observed when the extensive insertion/deletion heterozygosity at this locus was eliminated, we conclude that the *CYC7-H3* sequences are inherently capable of acting as donors or recipients in gene conversion events, although they are unlikely to act as donors when they are located opposite a large heterology. DNA sequence comparisons revealed similarities between the *CYC7-H3* junction region and the 2-μm circle DNA region that is involved in site-specific recombination.

IN *Saccharomyces cerevisiae*, the length of DNA per genetic map unit averages 3 kb/cM for each chromosome (STRATHERN *et al.* 1979; SCHWARTZ and CANTOR 1984). Physical and genetic distances have been compared in several segments of the yeast genome, leading to the conclusion that many genomic intervals experience "average" levels of genetic exchange during meiosis (SHALIT *et al.* 1981; JACKSON and FINK 1985), although regions with lower than average levels (PETES 1979; LARKIN and WOOLFORD 1983) and higher than average levels (NASMYTH and REED 1980) have been reported. This relative uniformity of meiotic recombination frequency between genes could be a consequence of a uniform probability of initiating a recombination event between any two nucleotides, or it could reflect the relatively uniform distribution of specific sites that initiate recombination. Evidence for specific initiation sites in yeast is largely indirect and has been provided by observations of aberrant segregation of alleles at heterozygous loci, in which ratios other than the expected 2:2 ratio within a tetrad were obtained. After considering the frequencies of such gene conversion events and associated reciprocal ex-

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changes, it was concluded that all meiotic crossing over occurs by a mechanism that can lead to gene conversion (HURST, FOGEL and MORTIMER 1972; FOGEL *et al.* 1978). Conversion frequencies appear to depend on the position of an allele within the gene (LISSOUBA *et al.* 1962), because alleles close to one end of the gene are involved in gene conversion events more often than those lying further away. The simplest explanation for the observed gradient of conversion frequencies is to suppose that alleles with high conversion frequencies lie closer to a fixed initiation site for recombination than do alleles with low conversion frequencies (for review, see WHITEHOUSE 1982). Molecular characterization of alleles with high conversion frequencies and their surrounding DNA may allow the identification of sites that can initiate recombination.

Mutations which appear to have created new initiation sites for recombination have been described in *Schizosaccharomyces pombe* (GUTZ 1971) and in *Sordaria brevicollis* (MACDONALD and WHITEHOUSE 1979). These mutations (called M26 and YS17, respectively) showed an unusually high frequency of gene conversion that was associated with crossing over in the vicinity; furthermore, they showed a striking asymmetry in direction of gene conversion, in that they appeared to act in order to promote their own correction. These properties suggested that these sites act as recipients, not donors, of genetic information when involved in conversion events. MCKNIGHT, CARDILLO and SHERMAN (1981) reported that the *CYC7-H3* mutation in yeast showed disparity in the direction of gene conversion. This mutation, a 5-kb deletion, causes overproduction of iso-2-cytochrome *c*. The mutation also appeared to promote its own correction during gene conversion. Since most yeast alleles show approximate parity in the direction of gene conversion (FOGEL *et al.* 1978), we have undertaken additional analysis of disparity in gene conversion events involving *CYC7-H3* sequences. We have asked if cloned copies of *CYC7-H3* DNA introduced into the genome by transformation also show disparity in gene conversion. In addition, we have asked if the presence of the large insertion/deletion heterozygosity influences the direction of gene conversion events controlled by the *CYC7-H3* sequences.

MATERIALS AND METHODS

Tetrad analysis: The genotypes of haploid strains used in this study are given in Table 1. Diploid strains were isolated, sporulated and dissected, and the resulting haploid meiotic products were analyzed according to conventional procedures (SHERMAN and LAWRENCE 1974). Media appropriate for scoring the auxotrophic markers and drug-resistant markers segregating in these crosses were prepared according to MCKNIGHT, CARDILLO and SHERMAN (1981). Mating types were scored by spotting on tester strains with complementing auxotrophic markers (KZ8-5C and KZ8-1D for segregants of E726; KZ291-R20 and KZ91-R4 for the remaining crosses) and replicating onto minimal medium to detect prototrophs. Iso-2-cytochrome *c* levels were measured in intact cells using the spectroscopic method of SHERMAN and SLONIMSKI (1964). Since all strains lacked iso-1-cytochrome *c*, the intensity of the c_α band absorbance could be used to score the *CYC7* genotype. *CYC7-H3* or *CYP3-15* strains had strong c_α bands, *CYC7*$^+$ strains had weak c_α bands and *cyc7-28* or *cyc7-H3-28* strains had no c_α bands. The deletion that causes overproduction of iso-2-cytochrome *c* included two closely linked genes (*ANP1* and *RAD23*). Sensitivity to 2-amino-1-[*p*-nitrophenyl]-1,3-propane-

TABLE 1

Yeast strains

Strain no.	Genotype
E458-5B	*MATα cyc1 cyc7-H3-28 ura3 trp1*
E480-1D	*MATa cyc1-363 cyc7-28 ura3-52 leu2-3,112*
E480-P131-1	*MATa cyc1-363 cyc7-28 CYC7-P131*[a] *can1 ura3-52 leu2*
E639-1A	*MATα cyc1 ura3 CYC7*$^+$ *anp1-1 his1*
GM105-31B-1	*MATα cyc1 CYC7-H3 can1 his1 trp2*
GM105-86A	*MATa cyc1 CYC7-H3 trp2 lys2 his1*
KZ8-5C	*MATa cyc1-1 CYC7*$^+$ *his4 ura1*
KZ8-1D	*MATα cyc1-1 CYC7*$^+$ *his4 ura1*
KZ291-R20	*MATa cyc1-1 CYC7-H3-45-x can1 his5*
KZ91-R4	*MATα cyc1-1 CYC7-H3-45-x can1 his5*
PY5-10A	*MATa cyc1 CYC7-H3 lys2*
PY5-27C	*MATα cyc1 CYC7-H3 lys2*
PY5-25B	*MATa cyc1 ura3 cyc7-H3-28 lys2*
VP160-4D	*MATα cyc1 CYP3-15*[b] *his1 metX*

[a] Genotype of integrated plasmid pAB48 is *URA3*$^+$ *CYC7-H3 URA3*$^+$ *cyc7-H3-28.*
[b] *CYP3* and *CYC7* both refer to the gene which codes for iso-2-cytochrome *c.*

diol (ANP) was scored in all appropriate crosses. Sensitivity to ultraviolet radiation was scored in haploid members of convertant tetrads unless more than one *rad* gene was segregating in the cross. For such crosses, the haploid members of convertant tetrads were mated to *rad23* tester strains (PY5-10A or PY5-27C), and radiation sensitivity was scored in diploids as described (McKNIGHT, CARDILLO and SHERMAN 1981).

Recombinant DNA and yeast transformation procedures: The 4.6-kb yeast fragment from pAB38 that contains the *CYC7-H3* gene (McKNIGHT, CARDILLO and SHERMAN 1981) was inserted into the *Hin*dIII site of YIp5 (STRUHL *et al.* 1979). The resulting plasmid (pAB48) was linearized by partial *Hin*dIII digestion and was used to transform strain E480-1D (ERREDE *et al.* 1984). Plasmid pAB25 is described in McKNIGHT, CARDILLO and SHERMAN (1981). Procedures for yeast DNA isolation, restriction endonuclease digestion of genomic DNA and hybridization with cloned fragments for analyses of parental and convertant chromosomes are detailed in ERREDE *et al.* (1984).

RESULTS

Gene conversion events involving *CYC7-H3* sequences at an altered position in the genome: McKNIGHT, CARDILLO and SHERMAN (1981) observed ten tetrads among the 294 they examined in which the *CYC7-H3* regulatory region showed 1:3 segregation. They failed to observe any tetrads in which this sequence showed a 3:1 segregation pattern. To determine if such disparity in gene conversion of the *CYC7-H3* sequence was an autonomous property of this sequence or if the disparity resulted from a more subtle position effect at this region, and to begin to delimit the sequences responsible for this unusual asymmetry in gene conversion, we made use of a cloned copy of the *CYC7-H3* gene (pAB48) that includes 5 kb of yeast DNA centered around the *CYC7-H3* gene. This plasmid was used to transform strain E480-1D, and a transformant, E480-P131-1, with a duplication of this 10.6-kb plasmid was selected for study. The physical organization of the *CYC7* region, summarized in Figure 1, was

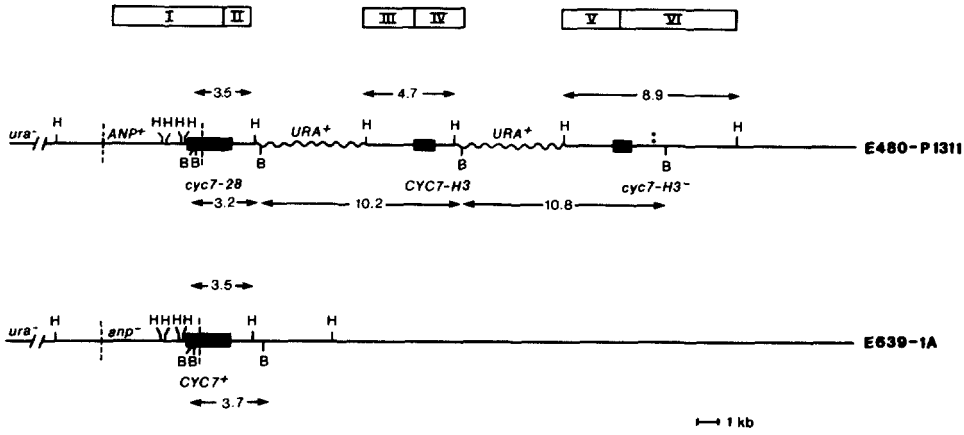

FIGURE 1.—Map of restriction enzyme recognition sites in the *CYC7* region that were used to analyze gene conversion events in cross E726. The wavy lines represent vector sequences and the boxes represent regions homologous to the *CYC7*$^+$ probe. The sizes of the *Hin*dIII (H) fragments and of the *Bam*HI (B) fragments that hybridize to this probe are indicated above and below the maps, respectively. The dashes and roman numerals refer to regions described in the text.

deduced from restriction enzyme digestion and probing with a 2.2-kb *Eco*RI fragment containing the *CYC7*$^+$ gene purified from plasmid pAB25. Sequences homologous to this *CYC7*$^+$ probe are indicated with black boxes in Figure 1. The recipient strain, E480-1D, lacked a *Hin*dIII site immediately 3′ to the *CYC7* region (region VI, Figure 1), a site that is present in other strains. Also, *CYC7-H3* sequences lack *Hin*dIII sites immediately 5′ to the native gene because the *CYC7-H3* mutation resulted from a deletion of 5 kb of 5′ flanking sequence (delimited by dashed lines in region I, Figure 1). Thus, the *CYC7*$^+$ probe hybridizes to a 7.6-kb *Hin*dIII fragment in strain E480-1D, to a 3.5-kb *Hin*dIII fragment in other strains with wild-type or point mutant *CYC7* alleles and to a 4.7-kb fragment in *CYC7-H3* strains. When E480 P131-1 was digested with *Hin*dIII and hybridized with the *CYC7*$^+$ probe, hybridization to three fragments (8.9, 4.7 and 3.5 kb) was observed, identical to the pattern in Figure 2, lane c. The 8.9- and 3.5-kb fragments would result from a single integration into the 7.6-kb *Hin*dIII fragment of E480-1D. Since a 4.7-kb fragment was also obtained, we conclude that a second plasmid was integrated at this region as diagrammed in Figure 1. However, we never detected an amount of iso-2-cytochrome *c* that would be produced by two functioning *CYC7-H3* genes per cell. Since all losses of iso-2-cytochrome *c* overproduction in the convertant tetrads were associated with physical alterations at region III-IV (see below), we concluded that region III-IV contained the single functioning *CYC7-H3* gene in this transformant. Although it is likely that the *cyc7-H3*$^-$ allele in region V-VI is *cyc7-H3-28* (which could have arisen during, or subsequent to, the primary transformation event), we have designated this allele as *cyc7-H3*$^-$ because its precise origin is unknown. Thus, in the E480-P131-1 strain, the *CYC7-H3* regulatory sequences have been moved about 10 kb distal to their usual position, surrounded by foreign DNA and duplicated.

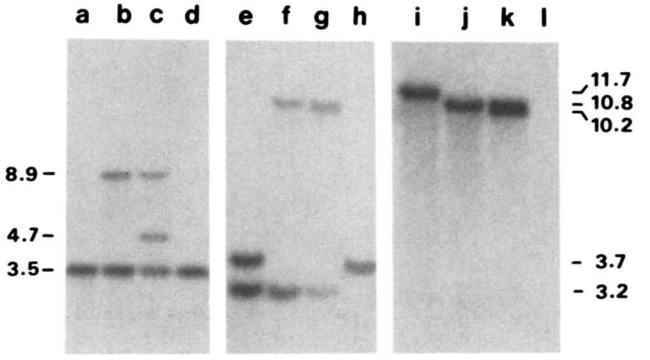

FIGURE 2.—Autoradiographic determination of sequences remaining after gene conversion (1:3 tetrad with crossover). Lanes a–d contain DNAs extracted from the four members of tetrad 121, which were digested with *Hin*dIII and probed with the *CYC7*$^+$ probe. Lanes e–h contain these same DNAs digested with *Bam*HI and hybridized with the *CYC7*$^+$ probe, and lanes i–l contain the same DNAs digested with *Bam*HI and hybridized with pBR322. Numbers to the left of the figure indicate sizes of *Hin*dIII fragments (in kilobases), and numbers to the right indicate sizes of *Bam*HI fragments. The Cyc7 phenotypes were c_α weak (a, e and i); c_α weak (b, f and j); c_α strong (c, g and k); c_α weak (d, h and l).

E480-P131-1 was crossed to E639-1A, and 185 tetrads from this diploid (E726) were analyzed. Despite the extensive insertion-deletion heterozygosity in this region, frequent reciprocal and nonreciprocal recombination occurred. We observed 14 reciprocal exchanges in region I and two reciprocal exchanges in region II (Figure 1) giving tetratype tetrads for *ANP1* and *URA3* (0.7 kb/cM), as well as six reciprocal exchanges in region IV that resulted in tetrads with three Ura$^+$ spores and one Ura$^-$ spore (0.5 kb/cM). There were 11 aberrant segregations at *CYC7* that were not associated with a reciprocal exchange, and there were three aberrant segregations at *CYC7* in which the convertant chromatid was also involved in a crossover, described above (Table 2).

In 13 of these 14 aberrant tetrads, two segregants were similar to the E639-1A parent and produced wild-type levels of iso-2-cytochrome *c* (c_α weak), but only one segregant was similar to the E480-P131-1 parent and overproduced iso-2-cytochrome *c* (c_α strong). There are three types of events that could cause loss of overexpression in the convertant segregant. First, loss of expression without gross sequence alteration could occur via an intrachromosomal gene conversion within the structural portion of the *CYC7-H3* gene (region IV). Second, wild-type levels of expression at the *CYC7-H3* locus could be restored by an interchromosomal gene conversion event in which insertion of the 5 kb of 5′ flanking sequence (delimited by dashed lines in Figure 1) occurred at the region III-IV boundary. Third, loss of the *CYC7-H3* sequences could occur by interchromosomal gene conversion, or intrachromosomal recombination. To distinguish between these possibilities, Southern analysis was performed. As described below, we failed to observe the first type of event, but we did observe both the second (restoration of the wild-type regulatory region at the *CYC7-H3* locus) and the third (loss of *CYC7-H3* sequences).

TABLE 2

Aberrant segregation of *CYC7* alleles

Diploid	Alleles	Segregation pattern[a]							
		2:2	3:1	1:3	2:2:0	1:2:1	1:3:0	2:1:1	3:1:0
E726 (E480-P131-1 × E639-1A)	*CYC7-H3:CYC7⁺:cyc7⁻*				170	8	5	1	0
PY1 (E480-1D × GM105-31B-1)	*CYC7-H3:cyc7-28*	104	0	1					
PY5 (GM105-86A × E458-5B)	*CYC7-H3:cyc7-H3-28*	77	7	3					
PY12 (VP160-4D × PY5-25B)	*CYP3-15:cyc7-H3-28*	189	8	7					

[a] For each cross, these ratios refer to the alleles in the order listed.

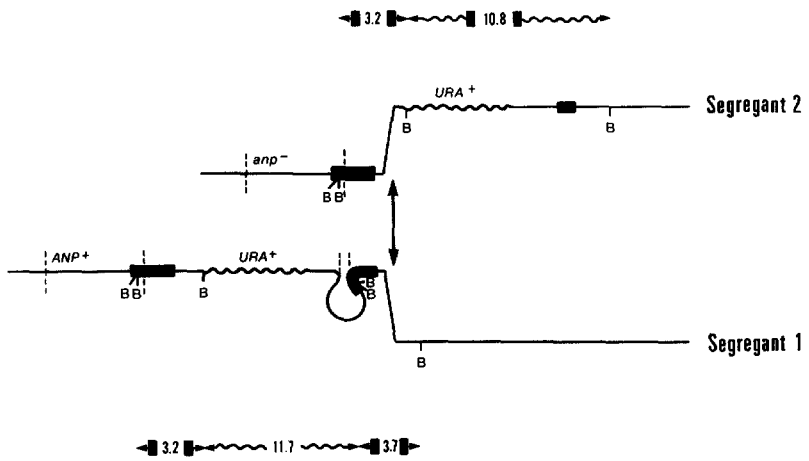

FIGURE 3.—Diagram of recombinant segregants from tetrad analyzed in Figure 2. *Bam*HI fragments hybridizing to the *CYC7*$^+$ probe are indicated with boxes; those hybridizing to the pBR322 probe are indicated with wavy lines. The arrow between the two chromatids indicates the position of the crossover.

We failed to find evidence for intrachromosomal gene conversion. In all 13 tetrads, DNA extracted from the aberrant segregant contained only two *Hin*dIII fragments (8.9 and 3.5 kb) that hybridized to the *CYC7*$^+$ probe (illustrated in Figure 2, lane b; Figure 4, lane a). The 4.7-kb fragment (region III-IV) was altered in size or missing in every case. These aberrant segregations were not the result of simple intrachromosomal conversion of the structural portion of the *CYC7-H3* gene, because if such an event had occurred, three *Hin*dIII fragments (8.9, 4.7 and 3.5 kb) should have remained. This was a surprising result because others had shown that intrachromosomal gene conversion is a frequent cause of aberrant segregation in unselected tetrads carrying nontandem duplications (KLEIN and PETES 1981; KLEIN 1984; JACKSON and FINK 1985).

In one of the aberrant tetrads, we observed a pattern consistent with insertion of *CYC7*$^+$ regulatory sequences at the region III-IV boundary. In addition, a crossover in region IV had occurred. The Southern blots that led to this interpretation are shown in Figure 2, and a diagram of the chromatids that resulted from the interchromosomal gene conversion and crossover is shown in Figure 3. In this tetrad, segregant 3 was similar to the E480-P131-1 parent (c_α strong, Anp$^+$, Ura$^+$) and segregant 4 was similar to the E639-1A parent (c_α weak, Anp$^-$, Ura$^-$). Segregant 2 was a c_α weak, Anp$^-$, Ura$^+$ recombinant, and segregant 1 was a c_α weak, Anp$^+$, Ura$^+$ convertant. The occurrence of the conversion event and the crossover was demonstrated by digesting DNAs from these segregants with *Bam*HI and probing with both the *CYC7*$^+$ probe (Figure 2, lanes e–h) and with pBR322 sequences (Figure 2, lanes i–l). As shown in Figure 2 and diagrammed in Figure 3, segregant 2 has a 10.8- and a 3.2-kb fragment, both of which hybridize to the *CYC7*$^+$ probe, and a 10.8-kb fragment that hybridizes to the pBR322 probe. Segregant 1 has an 11.7-kb fragment that hybridizes to the pBR322 probe. This is the size predicted following

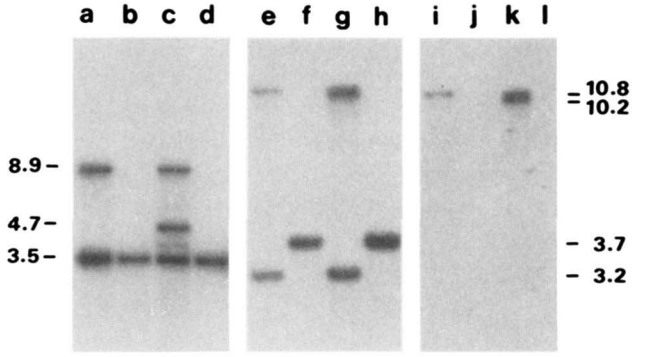

FIGURE 4.—Autoradiographic determination of sequences remaining after gene conversion (1:2:1 tetrad). Lanes a–d contain DNAs extracted from the four members of tetrad 171; these were digested with *Hin*dIII and hybridized with the *CYC7*$^+$ probe. Lanes e–h contain these same DNAs digested with *Bam*HI and hybridized with the *CYC7*$^+$ probe, and lanes i–l contain the same DNAs digested with *Bam*HI and hybridized with pBR322. Numbers to the left of the figure indicate sizes of *Hin*dIII fragments (in kilobases), and numbers to the right indicate sizes of *Bam*HI fragments. Minor bands in lane c result from partial digestion products. The Cyc7 phenotypes were c_α^- (a, e and i); c_α weak (b, f and j); c_α strong (c, g and k); c_α weak (d, h and l).

insertion of the E639-1A sequences delimited by the dashed lines. Segregant 1 also has a 3.2- and a 3.7-kb fragment, both of which hybridize to the *CYC7*$^+$ probe.

In another tetrad with only a single *CYC7-H3* segregant, the event could also be attributed to interchromosomal gene conversion. In this case, co-conversion across the entire region had occurred. This tetrad had one c_α strong, Anp$^+$, Ura$^+$ segregant (similar to E480-P131-1) and three c_α weak, Anp$^-$, Ura$^-$ segregants (similar to E639-1A). DNA from three of the segregants (including the aberrant segregant) contained only a 3.5-kb *Hin*dIII fragment that hybridized to the *CYC7*$^+$ probe and did not hybridize to pBR322 sequences, indicating that a conversion event from a point proximal to the *ANP1* locus to a point in region VI distal to the "missing" *Hin*dIII site in E480-P131-1 had occurred (results not shown). There is no simple intrachromosomal recombination event that could give rise to a c_α weak, Anp$^-$, Ura$^-$ segregant with only this 3.5-kb *Hin*dIII fragment remaining.

In the remaining 11 tetrads with only a single *CYC7-H3* segregant, we concluded that the *CYC7-H3* sequences in region III-IV had been removed. The sizes of the *Hin*dIII and *Bam*HI fragments that hybridize to *CYC7* or pBR322 probes are shown in Figure 4. The aberrant segregant was missing a 4.7-kb *Hin*dIII fragment that hybridized to the *CYC7*$^+$ probe (lane a), as well as a 10.2-kb *Bam*HI fragment that hybridized to the *CYC7*$^+$ probe (lane e) and to the pBR322 probe (lane i). In eight of these 11 tetrads, the aberrant segregant was c_α^- (due to the loss of the *CYC7-H3* sequence in region III-IV). In two of these eight, the "convertant" chromatid was also involved in a reciprocal exchange in region I (between *ANP1*$^+$ and *cyc7-28*). In three of the 11 tetrads, the aberrant segregant was c_α weak (due to the loss of *CYC7-H3* and the gain of *CYC7*$^+$). Although sequence loss resulting from intrachromosomal recom-

bination or interchromosomal gene conversion could not be distinguished using Southern blots, it seems unlikely that intrachromosomal reciprocal exchange was responsible for all these aberrant tetrads. To obtain a c_α weak segregant from a simple reciprocal exchange between region II and region IV, it would have to occur within a 339 nucleotide segment bounded by the *CYC7-H3* right-hand deletion breakpoint at position −222 (KOSIBA *et al.* 1982) and the *CYC7-28* mutation at amino acid 39 (unpublished data of T. CARDILLO, cited in ERREDE *et al.* 1984). It is unlikely that these aberrant tetrads arose by unequal sister chromatid exchange, since we failed to observe the predicted excess of iso-2-cytochrome *c* that would result from two functioning *CYC7-H3* genes in the same cell. Thus, it appears likely that at least some of these aberrant segregants arose by interchromosomal gene conversion.

We also observed a single tetrad with two c_α strong, one c_α weak and one c_α^- segregants. Since the convertant spore contained only a 3.5-kb fragment that hybridized to the *CYC7*$^+$ probe, we conclude that it resulted from conversion of a *CYC7*$^+$ chromosome to *cyc7*$^-$, rather than from a reciprocal exchange in region V.

In conclusion, we observed frequent reciprocal exchange and loss of *CYC7-H3* regulatory sequences in these tetrads. In only a single case did the *CYC7-H3* chromatid appear to act as a "donor" of genetic information in the structural portion of the gene. We failed to observe even a single case of 3:1 segregation of the regulatory region of the *CYC7-H3* gene. We conclude that the unusual asymmetric recombination behavior of the *CYC7-H3* regulatory region appears to be retained by cloned copies reintroduced into the genome.

Conversion behavior of a similar regulatory mutation, *CYP3-15*: Since the *CYC7-H3* mutation is a deletion of 5 kb, the lack of parity we observed when this site was involved in gene conversion events could have resulted from the insertion/deletion heterozygosity at this locus, without direct involvement of the *CYC7-H3* allele. To learn whether the *CYC7-H3* sequences or the insertion/deletion heterozygosity, or both, were required to observe disparity in gene conversion at this locus, we made use of the *CYP3-15* mutation kindly provided by J. VERDIERE. This mutation also causes overproduction of iso-2-cytochrome *c* and also results from a deletion of about 5 kb of DNA 5′ to the coding region of the gene. From inspection of the published sequence of *CYP3-15* (MONTGOMERY *et al.* 1982) and the published and unpublished sequence of *CYC7-H3* (KOSIBA *et al.* 1982; F. SHERMAN and L. MELNICK, personal communication), we were able to compare the breakpoints of these two deletions (Figure 5). Nearly identical sequences are deleted in these two strains, in that the only differences involve 33 nucleotides at one end and 60 nucleotides at the other end of the deleted segment (hatched areas in Figure 5). In contrast to *CYC7-H3*, *CYP3-15* shows parity in gene conversion in crosses to *CYC7*$^+$ (VERDIERE and PETROCHILO 1979; J. VERDIERE, personal communication). The availability of the *CYP3-15* mutation allowed us to examine co-conversion of the *CYC7* structural gene sequences in the presence of the *CYC7-H3* sequences but in the absence of extensive insertion-deletion heterozygosity. We constructed the diploid strain PY12 (VP160-4D × PY5-25B) and examined 204

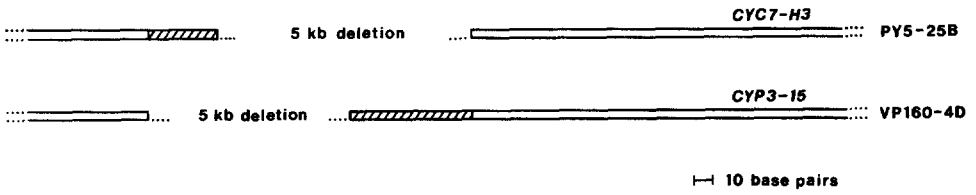

FIGURE 5.—Comparison of deletion breakpoints in *CYC7-H3* and *CYP3-15* mutations. The open portions indicate sequences retained in both mutations, and the cross-hatched portions indicate sequences retained in one mutation but not in the other.

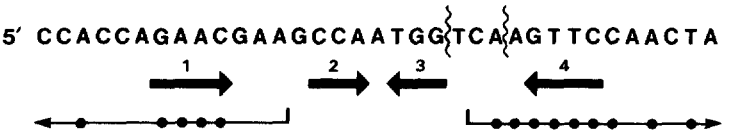

FIGURE 6.—Inverted repeats present near the *CYC7-H3* junction. The deletion breakpoint is between the wavy lines. The dots indicate homologies described in the text.

tetrads from this cross (Table 2). In this cross (*CYP3-15/cyc7-H3-28*), the regulatory regions are nearly identical (Figure 5), but conversion events in the *CYC7* structural gene can be scored. We observed 15 convertants among these 204 tetrads (7.3% ± 3.4%, 95% confidence limits). Although the *CYC7-H3* mutation was present on only one chromatid, parity in conversion frequency was seen. We conclude that to observe disparity in gene conversion at this locus, both *CYC7-H3* sequences and extensive insertion-deletion heterozygosity must be present.

We also observed a high frequency of gene conversion when the *CYC7-H3* regulatory sequences were homozygous. We examined 87 tetrads from the diploid PY5 (GM105-86A × E458-5B). In this cross, (*CYC7-H3/cyc7-H3-28*), the regulatory portion of the locus is homozygous, but conversion events involving the *cyc7-28* point mutation can be scored. We observed ten convertant tetrads, and both 3:1 and 1:3 segregations were recovered (Table 2). The frequency of gene conversion in PY5 was 11.5% ± 6.7% (95% confidence limits), and there was approximate parity in this cross. This frequency of gene conversion is significantly higher than that we observed in cross PY1 (GM105-31-B-1 × E480-1D), in which the junction sequences were heterozygous. In that cross (*CYC7-H3/cyc7-28*), we observed a single convertant tetrad (1% ± 2%; 95% confidence limits) in which co-conversion of the structural and regulatory regions had occurred (Table 2). These results suggest that the sequences at or near the *CYC7-H3* junction region that initiate or in some other manner control the frequency and direction of gene conversion in their vicinity are most active in the absence of insertion/deletion heterozygosity (PY5 and PY12) or in the presence of a second junction region on the same chromatid (E726).

Inspection of the DNA sequence immediately surrounding the deletion breakpoint in *CYC7-H3* revealed two blocks of inverted repeat sequences that were absent in the *CYP3-15* strain (Figure 6). The central inverted repeat (arrows 2 and 3) are flanked by an additional inverted repeat (arrows 1 and

4). This structure bears a striking similarity to that protected in yeast 2-μm circle DNA by the site-specific recombination protein, FLP (ANDREWS *et al.* 1985). In fact, the DNA sequence indicated by arrow 4 has nine of 13 nucleotides in common with the −16 to −4 element protected by FLP, and the DNA sequence indicated by arrow 1 has five of 11 nucleotides in common with the +3 to +15 element protected by FLP (these identities are indicated by dots in Figure 5). The central inverted repeat (arrows 2 and 3) has a similar size and structure to the 8-bp core region protected by FLP. Although the DNA sequence indicated by arrow 4 was also present at *CYP3-15* and at *CYC7*$^+$, there were no direct or inverted repeats in the proximal sequences; nor were there any unusual structural features immediately surrounding the *CYP3-15* breakpoint.

DISCUSSION

Diploid strains that are heterozygous for *CYC7-H3* and *CYC7* alleles show frequent gene conversion of a *CYC7-H3* allele, but not of the *CYC7* alleles (MCKNIGHT, CARDILLO and SHERMAN 1981). Furthermore, the frequency of gene conversion increases when the *CYC7-H3* regulatory sequences are made homozygous. These distinctive properties of the *CYC7-H3* regulatory sequences were retained by cloned copies reintroduced into the genome. The most common event we observed was the meiotic loss of *CYC7-H3* DNA sequences from a single chromatid. Southern blots cannot distinguish between intrachromosomal and interchromosomal origin for these segregants; however, the presence of three tetrads in which the aberrant chromatid was also involved in a reciprocal exchange suggests that these had an interchromosomal origin. The limited homology (339 nucleotides) available to generate a *CYC7*$^+$ segregant by intrachromosomal reciprocal exchange suggests that these tetrads also arose by interchromosomal gene conversion. The single tetrad in which the coconversion event was initiated proximal to the *ANP*$^+$ gene and terminated distal to the *CYC7*$^+$ gene (an interchromosomal conversion event involving more than 25 kb of DNA) suggests that there are no impediments to interchromosomal events involving extensive insertion/deletion heterozygosity in this region.

It is apparent that there is pronounced disparity in conversion frequency, with loss of *CYC7-H3* sequences being the predominant event, when this deletion is heterozygous. The *CYP3-15* deletion, although similar in size and location, does not show disparity in gene conversion when heterozygous. This result indicates that it is the unique *CYC7-H3* junction sequences that are responsible for the observed disparity, not the insertion/deletion heterozygosity *per se* or some undefined sequences proximal to the *ANP* locus. We have not tested whether the *RAD23* gene product is required to observe disparity in the direction of gene conversion at *CYC7-H3*. In the two *rad23*$^-$ diploids we examined (PY5 and PY12), parity was observed. However, it appears that the *RAD23* gene product is not sufficient for disparity at this location, since *CYP3-15* heterozygotes show parity.

When extensive insertion/deletion heterozygosity was absent, we observed frequent gene conversion and parity was restored, even when (as in PY12) the

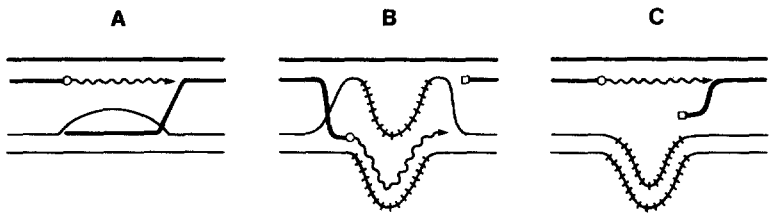

FIGURE 7.—Alternate modes of initiation of gene conversion at *CYC7-H3*. The solid lines of different thickness differentiate the two chromatids, and the circles indicate the positions of the initial nicks. The wavy lines indicate DNA synthesis extending from the 3′ end; the squares indicate exonucleases; and crosshatches indicate nonhomologous DNA. A, 5′ end donation; B, 5′ end degradation; C, failure of 5′ end donation to lead to gene conversion opposite a large heterology.

CYC7-H3 sequences were heterozygous. If most or all the conversion events in PY12 are initiated at *CYC7-H3*, then the initiating chromatid must be able to donate or receive information during gene conversion with equal probability. From a consideration of the genetic properties of the best characterized recombination "hot spot," the Chi sequence of *E. coli* (STAHL, LIEB and STAHL 1984; TAYLOR *et al.* 1985) and the biochemical properties of prokaryotic and eukaryotic proteins implicated in strand exchange (FLORY *et al.* 1984; RESNICK *et al.* 1984; KMIEC *et al.* 1984), it appears likely that a particular heterozygous initiating region will act as a genetic donor in some meioses and as a genetic recipient in others (Figure 7). The following model includes several relevant features from those cited above. We can imagine that the *CYC7-H3* sequences (circles) are nicked, leaving a 3′ hydroxyl group. If DNA synthesis is initiated from this 3′ hydroxyl and the displaced 5′ end "invades" the other duplex, then the initiating chromatid will act as a donor in the conversion event (Figure 7A). If, however, the 5′ end is degraded (squares) producing a gap, then the 3′ end might prime DNA synthesis from the homologous chromatid, displacing a single-strand loop that can repair the gapped initiating region (Figure 7B). The displaced loop might become double-stranded, as proposed by RADDING (1978); alternatively, the single-strand gap might be converted to a double-strand gap and be repaired, as proposed by SZOSTAK *et al.* (1983). In this fashion, the initiating region could promote its own correction. The presence of a large insertion opposite the initiating region might make 5′ end donation difficult, especially if the half-life of single-stranded DNA with a free 5′ end was relatively short due to exonucleases (Figure 7C). It has been assumed that the disparity in conversion frequency observed in YS17 of *S. brevicollis* (MACDONALD and WHITEHOUSE 1979) and in M26 of *S. pombe* (GUTZ 1971) reflected an inherent asymmetry in the initiation process *per se*, rather than a particular consequence of initiation at the base of an insertion heterozygosity, as is proposed here. The precise molecular nature of these mutations is unknown. These mutations may be associated with deletions in these genes that simultaneously create new recombination initiation sites and appear to act asymmetrically in a manner proposed for *CYC7-H3*. However, the ade$^-$ phenotype of M26 is suppressible by an extragenic suppressor, and YS17 has been observed to show postmeiotic segregation in altered genetic backgrounds. The 5′ strand

donation mode (Figure 7A) may be suppressed in these organisms, so that newly arising initiation sites act asymmetrically even in the absence of insertion-deletion heterozygosity.

Several site-specific nucleases, and their recognition sites, are known in yeast (KOSTRIKEN *et al.* 1983; WATABE *et al.* 1984; ANDREWS *et al.* 1985). We have compared the nucleotide sequence that flanks the deletion breakpoints in *CYC7-H3* to these published nuclease recognition sequences. There did not appear to be any significant homologies between either the *CYP3-15* or *CYC7-H3* junction sequences and the SCE I, SCE II or YZ endo recognition sequences. We observed limited sequence and structural homology between the *CYC7-H3* junction and sequences in 2-μm circle DNA that interact with the FLP recombinase during site-specific recombination events. The *CYC7-H3* junction may represent an unusual fortuitous creation of a FLP target sequence. Alternatively, the structural features shared by *CYC7-H3* and FLP may reflect common origin of general and site-specific recombination machines in yeast. It will be important to establish the role of these structural features in gene conversion at *CYC7-H3* and their occurrence at other sites in the yeast genome that appear to influence the frequency and outcome of gene conversion events in their vicinity.

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