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Concerted Deletions and Inversions Are Caused by Mitotic Recombination between Delta Sequences in *Saccharomyces cerevisiae*†

RODNEY ROTHSTEIN,* CYNTHIA HELMS,‡ AND NATHAN ROSENBERG

Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Received 24 September 1986/Accepted 11 December 1986

Deletions of a tyrosine tRNA suppressor gene, *SUP4*-o, are mediated by recombination between short repeated delta sequences in *Saccharomyces cerevisiae*. The arrangement of the five solo delta sequences that surround the *SUP4* locus was established by DNA sequence analysis. Seven deletion classes were identified by genomic blotting. DNA sequence analysis also showed that the delta sequences within a 6.5-kilobase region of the *SUP4* locus were the endpoints of these events. In three of these classes, an adjacent interval surrounded by delta sequences was inverted in concert with the deletion. The frequency of all deletion classes decreased in strains that contained mutations in the recombination and repair gene *RAD52*. We present two gene conversion mechanisms by which these rearrangements could have been generated. These models may also explain deletions between repeated sequences in other systems.

Genetic recombination between repeated sequences can lead to a variety of genomic rearrangements, including deletions, inversions, duplications, and translocations. In the yeast *Saccharomyces cerevisiae*, many of these events have the repeated sequence Ty or delta at their endpoints. Examples of such rearrangements include deletions of the *CYC1* gene (25) and deletion of the region between *HIS4* and *LEU2* (39, 40). In humans, recombination between repeated *Alu* sequences can likewise lead to deletions. Recently, several cases of familial hypercholesterolemia have been shown to be due to *Alu-Alu* recombination events that result in the loss of the C-terminal portion of the low-density lipoprotein (LDL) receptor (24). In this paper, we describe recombination events observed between delta sequences flanking a yeast tRNA gene and propose a mechanism to account for the deletions of the tRNA gene that may provide a general model for the generation of genomic rearrangements by recombination between repeated sequences.

Our earlier studies showed that deletions of a tyrosine tRNA suppressor, *SUP4*-o, occur at a relatively high frequency (1×10^{-7} to 3×10^{-7}) compared with other regions of the yeast genome (44). We found two size classes among 16 independent spontaneous deletions and suggested that these deletions had their endpoints in the delta sequences that surround the region. In this paper, we identify seven deletion classes by genomic blots and show by DNA sequence analysis that these deletions indeed have their endpoints in delta sequences. In addition, we find that most of the deletions probably result from gene conversion. These deletions are dependent on the pleiotropic recombination and repair gene *RAD52*. A functionally wild-type *RAD52* gene is necessary for several recombination-related functions, including the repair of X-ray damage (37), HO-catalyzed interconversion of the mating type (27), mitotic gene conversion (20, 35), meiosis (12), plasmid gap repair (32), and certain reciprocal exchange events (15). Observations of events following *RAD52*-dependent plasmid gap repair have led to a model for gene conversion postulating a double-strand break as the initiating lesion (52). Features of this model are used to explain the generation of deletions at the *SUP4* locus.

MATERIALS AND METHODS

Yeast and bacterial strains and genetic methods. The following yeast strains were used in this study: 332RW88-2B (*MAT***a** *SUP4-o ade2-1 lys2-1 trp5-2 leu1-12 can1-100 ura3-1* [44]); W87-4B (*MAT***a** *ade2-1 lys2-1 trp5-2 leu1-12 can1-100 ura3-1* [48]); W301-30A (*MAT***a** *SUP4-o ade2-1 his3-11,15 can1-100 ura3-1 leu2-3,112* [this study]); W342-3D (*MATα SUP4-o ade2-1 lys2-1 trp1-1 trp5-2 leu1-12 can1-100 ura3-1* [this study]); W301-38A (*MATα SUP4-o ade2-1 trp1-1 his3-11,15 can1-100 ura3-1 rad52-1* [this study]); and W338-6D and W338-8D (*MATα SUP4-o*::*URA3*$^+$ *ade2-1 trp1-1 his3-11,15 can1-100 ura3-1 rad52-1 trp5-2*? [this study]). Strains J57, J70, and J81 (44) are derivatives of 332RW88-2B that contained the point mutations *sup4-o-1*, *sup4-o-14*, and *sup4-o-25*, respectively, and were used to genetically define deletions. Of the strains listed in Table 2, J65, J69, J73, J74, and J80 were isolated from 332RW88-2B as described previously (44). J295 contains the deletion *sup4-o-130*, J244 contains the deletion *sup4-o-79*, and J330 contains the deletion *sup4-o-165*. The three were isolated from W342-3D, U400, and W301-30A, respectively, which all contain the *SUP4-o*::URA3$^+$ construction described below. The remaining 60 deletions that were analyzed by genomic blots were derived from 332RW88-2B, U400, or its derivatives. The *rad52-1* allele was scored by exposing freshly replica-plated cells to 50 krads of ionizing radiation with either a GE Maximar-100 at 100 kVP and 5 mA with no filter or a Gammacell-40 cesium source. The allele was introduced into the U400 background by crosses to create W338 and W339. Both *rad52-1* and *RAD*$^+$ segregants from these diploids were screened for deletions. The two *rad52-1* deletion strains J255, which contains *sup4-o-90*, and J256, which contains *sup4-o-91*, were isolated from W338-6D and

* Corresponding author.
† Dedicated to the memory of Giampiero di Mayorca.
‡ Present address: Collaborative Research, Inc., Bedford, MA 01730.

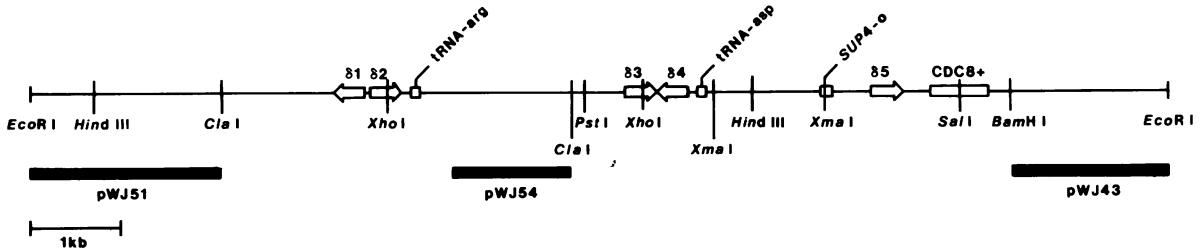


FIG. 1. Map of the *SUP4* region. The five delta sequences in the *SUP4* region are shown as large open arrows and are numbered consecutively from the leftmost *Eco*RI site. These numbers differ from those assigned by Gafner et al. (10) since their numbering system was based on the supposition that a sixth delta, inferred from electron microscopic data (3), exists near delta 2. Since this supposition is incorrect, we have numbered the deltas in this region by their actual occurrence in the genome. The orientation of the arrow reflects the direction of transcription initiated in deltas that are associated with Ty elements. The positions of the three tRNA genes and the *CDC8* gene are shown as boxes. The solid bars underneath the map represent the probes used for the genomic blot shown in Fig. 3.

W338-8D, respectively. Standard genetic manipulations of yeast were carried out as described in Sherman et al. (49).

The bacterial strain used for routine cloning and allele rescue was SF8 (4). Standard bacterial media and growth procedures were used (31).

DNA preparations from bacteria and yeast. Plasmid DNA from bacteria was isolated by the boiling procedure of Holmes and Quigley (18) to prepare the lysate. The cleared lysate was either centrifuged to equilibrium in CsCl in the presence of ethidium bromide or directly purified essentially as described previously (18).

Yeast DNA was purified from 10-ml cultures to yield on average 5 μg of DNA (5, 47), and 0.5 μg of this DNA was digested with restriction enzymes for genomic blots following the recommendations of the manufacturers. The yeast DNA isolated in this way was also used for allele rescue of the deletions for sequence analysis as described in a later section.

DNA sequence analysis. End-labeled restriction fragments for DNA sequence analysis were prepared by standard methods (28). Single end-labeled fragments were gel purified and sequenced by the method of Maxam and Gilbert (29). The sequence shown in Fig. 2 was determined by sequencing both strands of the parental strain. Recombinant deltas were determined for the most part by sequencing only one strand and using the parental sequence as a guide. Homology searches were performed with the FASTN program based on FASTP (26) at *k*-tuple = 1.

In addition to *SUP4*, three other genes have been identified in the region. The positions of the single-copy, essential arginyl tRNA gene near delta 2, the aspartyl tRNA near delta 4 (10), and the yeast thymidylate kinase gene, *CDC8* (1, 23), are shown in Fig. 1 and 2. Our *CDC8* DNA sequence differs from one published sequence by a threonine-to-serine change of the predicted protein sequence at amino acid 57 (1). It differs from the other published sequence at five positions, one resulting in the conservative aspartic acid-to-glycine substitution at position 188, while the remaining four result in silent third-position changes (23).

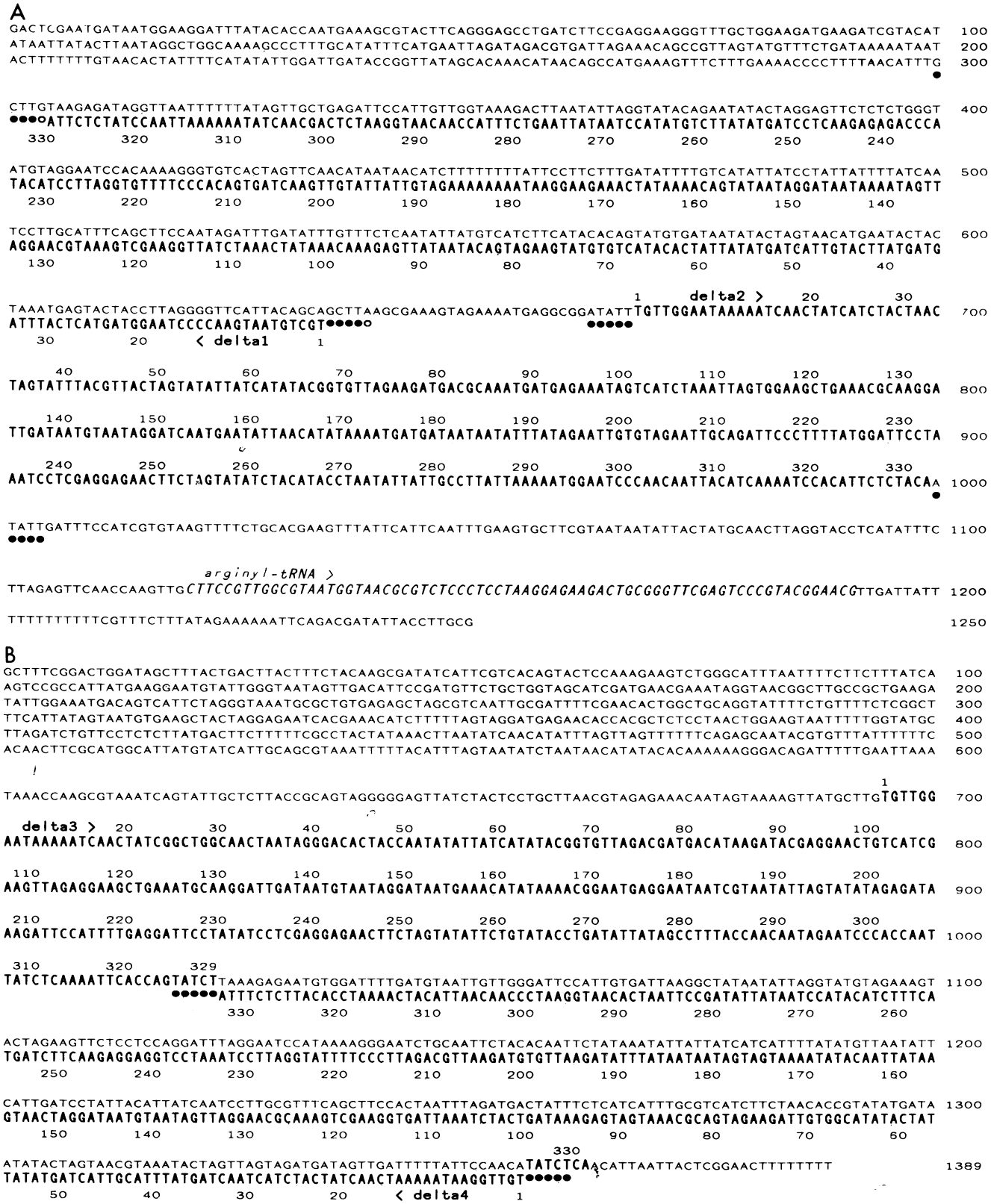
Rapid assay for *SUP4* deletions. To facilitate the identification of deletions of the *SUP4* locus, we used gene replacement methods to insert an additional marker, *URA3*$^+$, between deltas 4 and 5 near *SUP4* (45). An *Hin*dIII fragment containing the *URA3*$^+$ gene (42) was cloned into the *Hin*dIII site near the *SUP4*-o gene (Fig. 1). Next, the fragment containing this insertion was removed from the pBR322 vector by digesting with *Eco*RI and *Bam*HI. The linear DNA was used to transform W87-4B by the lithium chloride procedure of Ito et al. (19) and selecting for Ura$^+$ transformants. Transformants were next screened for suppressor function, which was assayed by suppression of *ade2-1*. DNA was isolated from several colonies that were simultaneously Ura$^+$ and Ade$^+$ to show by genomic blotting that the replacement had occurred in the *SUP4* region. This strain was called U400. The *SUP4* region is referred to as *SUP4-o*::*URA3*$^+$ to indicate that the *URA3*$^+$ gene is tightly linked to *SUP4-o*.

Strain U400 allowed us to distinguish deletions from point mutations by a simple replica-plating procedure. In this genetic background, loss-of-suppressor mutations are selected as canavanine-resistant colonies that are red as described previously (43). From 70 to 90% are point mutations in the tRNA gene, while the remainder are deletions. After replica-plating to medium lacking uracil, mutants carrying point mutations of the *SUP4* locus can grow, while those carrying deletions cannot due to the simultaneous loss of *SUP4-o* and *URA3*$^+$.

Hybridization procedures. Gel electrophoresis and hybridization of genomic blots of yeast DNA were performed as described previously (44) except that 0.5 μg of DNA was loaded per lane. The three plasmids, whose yeast inserts are represented by the bars in Fig. 1, were separately nick-translated (38) and simultaneously used as probes for the *SUP4* region. The conditions for hybridization were 65°C in 5× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0).

These same three plasmids were used to hybridize genomic blots at reduced stringency (60°C in 5× SSC). At this lower stringency, we expected to see bands on the blot that formed hybrids with any sequence that contained approximately 10% mismatched bases to the probe (2). We interpret the failure to observe any bands at this reduced stringency to mean that there is no significant homology to any of the multiple copies of delta sequences in the genome.

Allele rescue method. Techniques that facilitate the rcovery of chromosomal mutations have been described (see reference 47 for review). We chose a strategy described by Stiles et al. (50) that involves a two-step process. First, bacterial plasmid sequences are integrated adjacent to the desired region. In the next step, genomic DNA is isolated from the transformant, digested with an appropriate restriction enzyme, and ligated to fuse the desired adjacent sequence with the plasmid sequences. Plasmid pWJ88 was created by cloning the leftmost 2.1 kilobase (kb) *Eco*RI-*Cla*I fragment into the *Eco*RI and *Bam*HI sites of YIp5, which contains the *URA3*$^+$ gene (51). The plasmid was linearized at the unique *Hin*dIII site within the *Eco*RI-*Cla*I fragment and transformed into the various deletion mutant strains (32). To confirm that the integration event occurred at the desired


A
B
delta1
delta2
delta3
delta4
arginyl-tRNA

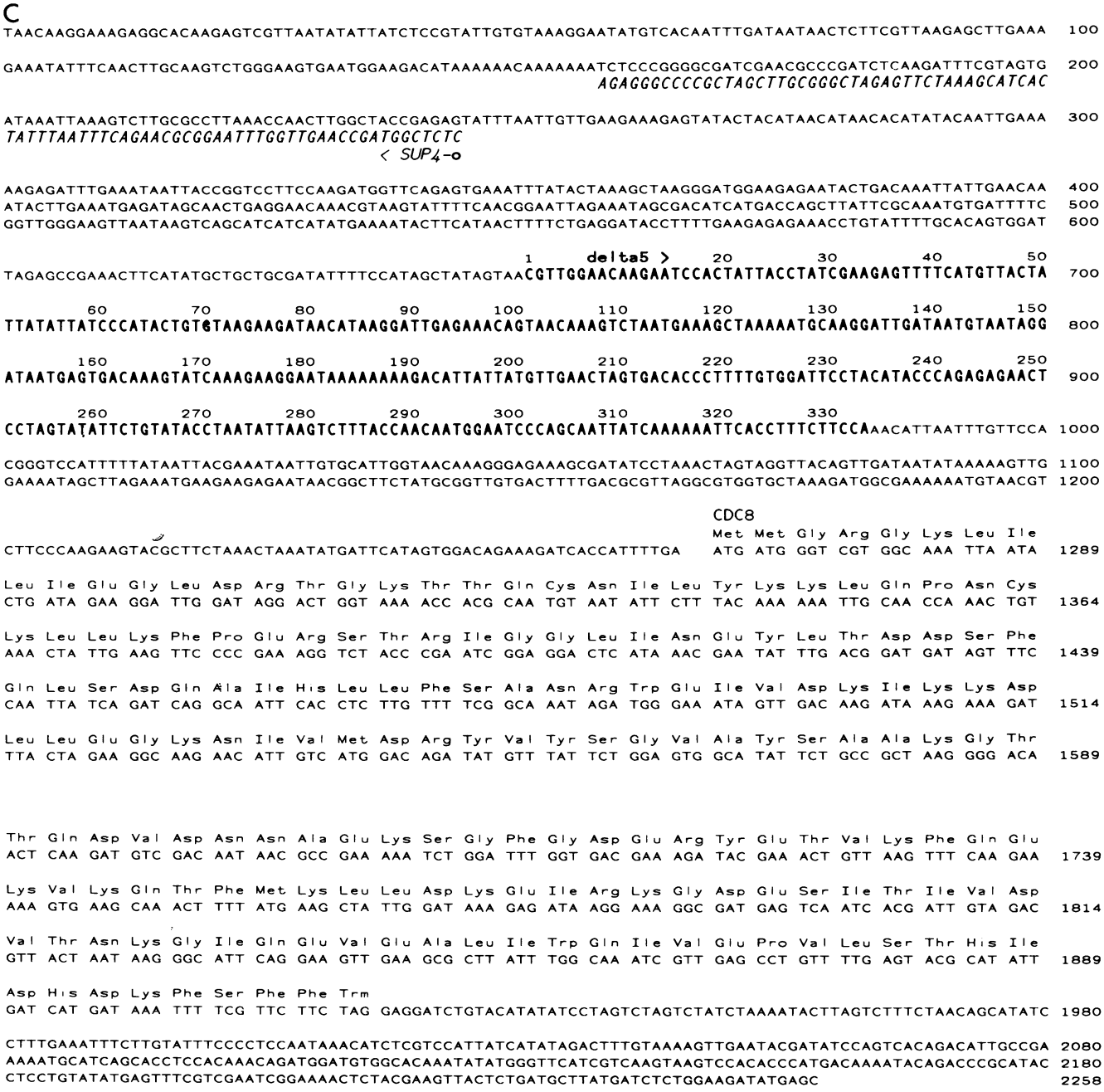


FIG. 2. Nucleotide sequence of the five deltas and adjacent genes in the *SUP4* region. The delta sequences in this region are found in both orientations. To facilitate later discussions, each delta sequence is numbered internally regardless of its orientation on the fragment and is printed in boldface type. For example, a delta sequence that is oriented 3′ to 5′ on the fragment is shown as a double-stranded sequence and is numbered on the bottom strand. (A) The sequence of delta 1 starts at nucleotide 634 and ends at 304. The target site duplication of the original Ty transposition is shown as five circles flanking both ends of delta 1. Four of the five circles are filled in, indicating identity. The 5′ end of delta 2 starts at nucleotide 668 and ends at 999. The nucleotides representing the target site duplication of delta 2 are indicated as filled-in circles. The arginyl tRNA gene, shown in italics, is encoded by the sequence from nucleotide 1120 to 1191. (B) Delta 3, which lies between nucleotides 695 and 1363, is interrupted at its 3′ end by delta 4. The 5-base-pair target site duplication flanking delta 4 is indicated by filled-in circles. Delta 4 lies between nucleotides 1024 and 1356. (C) The sequence of the *SUP4* ochre suppressor is shown in italics from nucleotide 158 to 244. Since it is transcribed from right to left, its sequence is given on the bottom strand. Delta 5 starts at nucleotide 651 and ends at 983. The open reading frame for the *CDC8* gene starts at 1266 and ends at 1916.

TABLE 1. Homology among the *SUP4* region deltas[a]

Delta element	% Homology			
	Delta 2	Delta 3	Delta 4	Delta 5
Delta 1	70.9	69.4	70.9	85.4
Delta 2		78.5	97.3	72.2
Delta 3			77.3	71.2
Delta 4				71.4

[a] Positions at which either sequence had an insertion or deletion were excluded from the comparison.

location, a genomic blot was performed on the transformants. The mutant *SUP4* region was rescued by digesting DNA from the transformant with *Sal*I, an enzyme that digests the DNA once in the vector sequence and once near the adjacent mutant sequence. The digest was cyclized with ligase, and ampicillin-resistant colonies were selected after transformation of the ligation mixture into competent *Escherichia coli* cells. Plasmid preparations from these transformants were used for DNA sequence analysis and further subcloning when needed.

RESULTS

DNA sequence analysis of the *SUP4* region. As a prerequisite to determining the precise rearrangements in the *SUP4* region, we determined the DNA sequence of the parental configuration of deltas surrounding the *SUP4* gene. The arrangement of the delta sequences in the *SUP4* region, originally described by Cameron et al. (3), was based on electron micrographs of single-stranded DNA secondary structure. We initiated our sequencing strategy with the electron microscope (EM) measurements as a guide and with the knowledge that delta sequences often contain *Xho*I restriction sites (9, 11) and that the *SUP4* gene contains an *Xma*I restriction site (13). In addition, partial DNA sequences of this region from related yeast strains were available (1, 10, 11, 23). We sequenced 4,897 nucleotides within the 12-kb *Eco*RI fragment containing the *SUP4* region. The positions and orientations of the five delta sequences on this fragment are shown in Fig. 1. The five solo delta sequences in the *SUP4* region were identified by searching for conserved characteristics found in published delta sequences (6). These conserved features include an *Hin*fI site at nucleotide 296, TGTTGGAATAA at the 5′ end, CA at the 3′ end, a total length of 333 nucleotides, and a 5-base-pair repeat of the chromosomal target site of the original Ty transposition. Conservation of these features among solo deltas throughout the genome most likely depends on the amount of evolutionary time since the transposition of the Ty element that contained the delta sequence.

Examination of the DNA sequence data from the *SUP4* region (Fig. 2) showed that delta 2 had all of the features common to delta sequences while the other four deltas exhibited some differences from the common sequence. For example, delta 4 ended with the dinucleotide TA instead of the more common CA. Delta 4 is most likely the result of a Ty transposition that invaded the 3′ end of delta 3 and subsequently deleted the central portion of the Ty element, leaving a solo delta. The duplication of the target site of delta 4 was accounted for in positioning the 3′ end of delta 3. Only four of five bases are conserved in the target site duplication of delta 1. Presumably the single C,T difference is the result of a transition mutation that occurred after the transposition event. Deltas 3 and 5 do not show the 5-base-pair duplication of the target site typical of delta sequences. One possible explanation is that these deltas participated in earlier recombination events, with the result that each delta now has two different target site duplications.

Table 1 shows the results of comparing the sequence relatedness of the five deltas. The range of homology shown by these sequences was similar to what has been reported for deltas found at various positions in the yeast genome (6, 9–11). The most homologous pairs of deltas are deltas 2 and 4 (97%) and deltas 1 and 5 (85% homologous).

Finally, we searched for the existence of additional deltas in the region, since a sixth delta sequence adjacent to deltas 1 and 2 was originally proposed from the electron microscopy study (3). None were found. First, we compared the 3,225 additional nucleotides surrounding the five deltas and did not find any homology to delta. Second, we showed that the three fragments indicated by bars in Fig. 1 contained no significant homologies to delta by probing genomic blots at lower stringency (60°C in 5× SSC).

Genomic blots reveal seven classes of deletion. In addition to the 16 deletions reported in our first publication (44), 54 more spontaneous deletions of *SUP4* were isolated. Among the new deletions, 18 were isolated and identified as loss of suppressor mutations that failed to recombine with three separate point mutations scattered throughout the gene (*sup4-o-1*, *-14*, and *-25* [44]). The remaining 36 were isolated by screening for simultaneous loss of *SUP4* and a *URA3*$^+$ marker that was inserted close to it in strain U400 and its derivatives (see Materials and Methods). Since the DNA sequence of the *SUP4* region showed that two of the five delta sequences contained *Xho*I sites, we decided that genomic blots of DNA digested with *Eco*RI and *Xho*I would yield the most information about the delta sequence rearrangements after deletion. DNA from all 70 of the genetically defined deletions was digested with both *Eco*RI and *Xho*I. In the parental strain, blotting this digest generated three bands after hybidizing simultaneously with the three unique probes shown in Fig. 1. We observed only seven distinct deletion classes (Fig. 3). Class II and IV, which have identical *Eco*RI and *Xho*I patterns, can be distinguished by an additional blot of an *Eco*RI-*Pst*I digest. A summary of the blot analysis is presented in the last column in Table 2.

Characterization of the seven deletion classes. The precise rearrangement of at least one representative from each of the seven deletion classes was revealed by DNA sequence analysis of the appropriate junction fragments from genomic clones isolated from each deletion strain. The majority (75%) of the deletions of *SUP4* are 2.1 kb in size (Table 2). The sequence analysis of these class I to IV deletions revealed that they were most likely generated by a gene conversion event in which information from the delta 1-2 region converted the region between deltas 4 and 5. The class I deletion J295 consisted of two recombinant deltas (Table 2). The event that generated the delta 1-5 recombinant occurred somewhere between nucleotides 121 and 138 of delta 1 and between nucleotides 124 and 141 of delta 5. The junction cannot be more precisely located since the two delta sequences are identical for 18 base pairs between the two polymorphisms that flank the recombination joint. The cross-over point in the delta 2-4 recombinant is likewise uncertain due to the identity of the first 239 nucleotides of these two deltas. Figure 4 illustrates the configuration of the class I and II deletions, each of which contain two recombinant deltas flanking the deleted region. In each case a delta 1-5 recombinant and a delta 2-4 recombinant are adjacent to each other, replacing the *SUP4*-containing fragment. This

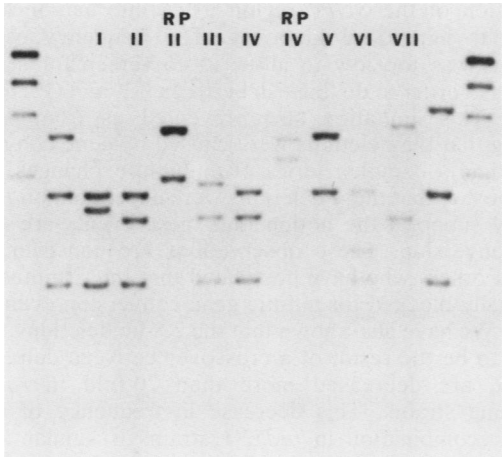


FIG. 3. Genomic blot of the seven deletion classes. A representative deletion from each class was examined by genomic blot. DNA isolated from two parental strains and each deletion strain was digested with *Eco*RI and *Xho*I, electrophoresed, and blotted to nitrocellulose filter paper. Class II and IV deletions were also digested with *Eco*RI and *Pst*I (lanes labeled RP) and treated in an identical manner. Blots were hybridized simultaneously with all three radiolabeled unique probes from the *SUP4* region, shown in Fig. 1. Subsequent autoradiography revealed the pattern shown above. The representative deletions are labeled by their class number. The lane adjacent to the class I deletion contains parental *SUP4* strain DNA, and the lane adjacent to the class VII deletion contains U400 DNA. The end lanes each contain bacteriophage lambda DNA digested with *Hin*dIII as a molecular weight standard.

gene conversion event results in the precise duplication of the 33 nucleotides between deltas 1 and 2. We also sequenced deltas 1 and 2 in this class I deletion and found that they were unchanged after this event. The class II deletions, J74 and J80, had similar recombinant delta arrangements, but the delta 2-4 recombinant contained the *Xho*I site of delta 2. This accounts for the shorter *Eco*RI-*Xho*I fragment in the genomic blots of class II deletions (Fig. 3).

Class III and IV deletions also contained two recombinant deltas, but their arrangement differed from class I and II deletions in that there was an adjacent inversion of the segment bounded by deltas 2 and 3 (Fig. 4). This was revealed by both genomic blotting and DNA sequence analysis. After digestion with *Eco*RI and *Pst*I, genomic blots of a class III deletion, J73, and a class IV deletion, J244, gave rise to two fragments with altered molecular weights compared with the other 2.1-kb deletions. The shift in the position of the *Pst*I site was caused by the inversion. Delta 2 was intact but because of the inversion was found adjacent to the delta 1 end of the delta 1-5 recombinant. A delta 2-4 recombinant adjacent to an intact delta 3 formed the other boundary of the inverted segment. In the class III deletions, the conversion event between deltas 2 and 4 did not include the *Xho*I site of delta 2, while in the class IV deletions the event included the *Xho*I site of delta 2 (Table 2). If the inversion were an independent event, we would have expected to find an inversion in wild-type strains at the same frequency as in the deletions (12 of 52). Since genomic blots of wild-type strains do not reveal inversions occurring at that frequency, we conclude that the inversion occurs as a concerted event with the deletion.

The remaining three classes (V, VI, and VII) each had a 2.8-kb deletion, which equaled the distance between deltas 3 and 5. The DNA sequence of the recombinant junctions in several 2.8-kb deletion strains showed that direct repeat recombination between these two deltas had generated the deletions. The recombinant delta that resulted from a class V event contained the *Xho*I site found in delta 3. In the case of a class VI deletion, the *Xho*I site of delta 3 was lost due to the position of the crossover (Table 2). The last deletion class, VII, also contained an inversion of the adjacent region similar to that described for the class III and IV events. Genomic blots of a class VII deletion with *Eco*RI and *Pst*I

TABLE 2. Crossover positions of the recombinant deltas[a]

Deletion class	Strain	Recombinant delta (position of crossover)	No. in class[b]
I	J295	δ1 (121–138)/ δ5 (124–141)	35
		δ2 (1–239)/ δ4 (1–239)	
II	J74	δ1 (38–49)/ δ5 (39–50)	5
		δ2 (291–328)/ δ4 (292–329)	
	J80	δ1 (141–152)/ δ5 (144–155)	
		δ2 (291–328)/ δ4 (292–329)	
III	J73	δ1 (316–319)/ δ5 (322–325)	6
		δ2 (1–239)/ δ4 (1–239)	
IV	J244	δ1 (162–187)/ δ5 (166–191)	6
		δ2 (291–328)/ δ4 (292–329)	
V	J65	δ3 (123–156)/ δ5 (124–157)	11
	J255*	δ3 (123–156)/ δ5 (124–157)	
VI	J330	δ3 (303–310)/ δ5 (305–312)	5
	J256*	δ3 (249–268)/ δ5 (252–271)	
VII	J69	δ1 (143–154)/ δ3 (141–152)	2
		δ1 (189–295)/ δ5 (194–300)	

[a] The position of the crossover for each recombinant delta is given as a range that reflects the distance between the polymorphisms that mark the recombination interval. The class V and VI deletions marked with an asterisk are the two deletions recovered in *rad52* strains.

[b] Number of deletions found in each class among the 70 deletions analyzed. These were classified by their blot pattern; their exact nucleotide crossover point has not been determined.

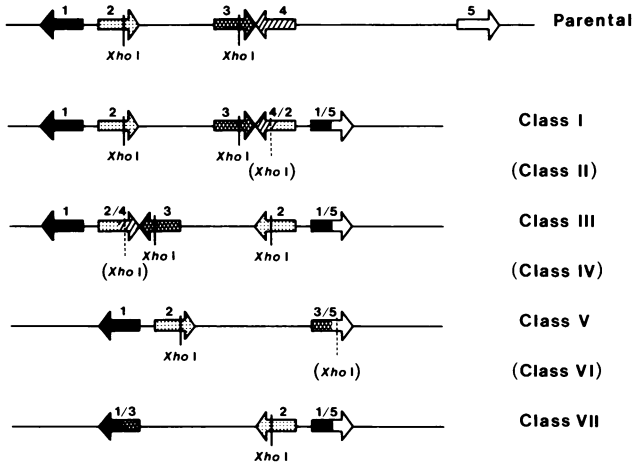


FIG. 4. Arrangement of recombinant deltas in the seven deletion classes. The different deltas are indicated by different patterns inside the arrows. Class I differs from class II by the appearance of the *Xho*I site in the recombinant delta 2-4 of class II. The position of the *Xho*I site in the class II deletion is indicated in parentheses in the figure. The same *Xho*I site difference occurs between class III and IV, and the position is indicated in parentheses. The difference between class V and class VI is the presence of the *Xho*I site in the delta 3-5 recombinant (also indicated in parentheses). The class VII deletion-inversion contains only one *Xho*I site in delta 2. The deltas in the figure are not drawn to scale. The precise rearrangement of the recombinant deltas from at least one representative of each class can be found in Table 2.

gave rise to two fragments with altered molecular weights compared with the other 2.8-kb deletions (data not shown). As was the case for the class III and IV deletions, the shift in the position of the *Pst*I site was caused by the inversion. DNA sequence analysis of the recombinant delta junctions revealed that delta 2 was intact but because of the inversion was found adjacent to the delta 1 end of the delta 1-5 recombinant. A delta 1-3 recombinant formed the other boundary of the inverted segment (Fig. 4).

***rad52-1* mutation affects spontaneous deletion frequencies at *SUP4*.** We next investigated the effect of the pleiotropic recombination and repair-deficient mutation *rad52-1* on deletions in the *SUP4* region. Since it has been postulated that *rad52-1* blocks mitotic gene conversion but does not affect direct repeat recombination (20, 34, 56), we were interested in investigating the deletion classes that arise in a *rad52-1* background. To minimize the problems of comparing strains with different genetic backgrounds, we chose three wild-type and four *rad52-1* strains that were closely related. Each of these strains contains *URA3*$^+$ linked to *SUP4* to permit the detection of deletions by a replica-plating assay. In the wild-type segregants, deletions occur at a frequency of 0.7×10^{-7} to 3×10^{-7}. Genomic blots of 18 deletions generated by these strains showed that the seven deletion classes were distributed similarly to that shown in Table 2. The frequency of suppressor loss in the *rad52* segregants was 1×10^{-5} to 4×10^{-5}. This was a 12- to 60-fold increase in point mutations compared with the wild type and was probably due to the hypermutable phenotype observed in *rad52* strains (35; M. Hoekstra and R. Malone, personal communcation). In sharp contrast to the wild type, the *rad52* segregants gave rise to only 2 deletions among the 24,115 loss-of-suppressor mutants that were screened, resulting in a deletion frequency of 0.8×10^{-9} to 3.3×10^{-9}. Genomic blots and subsequent DNA sequence analysis of the two strains recovered in *rad52-1* cells revealed that each was a delta 3-5 recombinant—one a class V and one a class VI deletion (Table 2). Therefore, the 2.1-kb classes occur at a frequency of less than 0.4×10^{-9} to 1.7×10^{-9}, and the direct-repeat deletion classes occur at 0.8×10^{-9} to 3.3×10^{-9} in the *rad52* strains. Since in wild-type strains the 2.1-kb deletion classes occur at a frequency of 53×10^{-9} to 225×10^{-9}, they are reduced more than 100-fold in *rad52*. The direct-repeat recombination deletion classes V and VI occur at a frequency of 17×10^{-9} to 75×10^{-9} in wild-type strains, and thus the absence of a functional *RAD52*$^+$ gene lowers the frequency of these deletions more than 20-fold. These class V and VI deletions could be the result of either intrachromosomal events or unequal sister chromatid exchanges between the directly repeated deltas.

DISCUSSION

In this paper, we have described the organization of the five solo delta sequences near the *SUP4* gene in *S. cerevisiae* and the types of genomic rearrangements that occur at this locus. Deletions, as well as deletions with associated inversions, were found, and both were the products of homology-dependent exchanges rather than illegitimate recombination, since recombinant intact deltas were produced. Certain classes of deletions were not found, such as delta 2-delta 5 deletions, presumably due to the essential arginyl tRNA gene between these deltas. The 2.1-kb classes (I to IV) were decreased more than 100-fold in strains containing a mutation in the pleiotropic recombination and repair gene *RAD52*. These classes (2.1 kb) appear to be due to gene conversion of the *SUP4* region using information from a delta-delta junction 3 kb away. The frequency of these deletions was too low to allow us to screen for sectored colonies in order to distinguish between G1- and G2-initiated events. This limitation also prevented us from directly showing that the deletions were caused by gene conversion rather than a complex series of multiple exchanges. However, the fact that these deletions were dependent on *RAD52* strongly supports the notion that these events are due to gene conversion. These observations are consistent with those of others who have postulated that *rad52* mutants are specifically blocked for mitotic gene conversion events (20, 27, 35). We have also shown that the 2.8-kb deletions, which appear to be the result of a crossover between delta 3 and delta 5, are decreased more than 20-fold in *rad52-1*-containing strains. This decrease in frequency of direct-repeat recombination in *rad52-1* strains is similar to that found for delta recombination flanking a Ty element (55) and is greater than that observed for other cases in which the *rad52-1* mutation decreases crossing-over between non-delta-containing direct repeats only 2- to 10-fold (20, 34, 56). In addition to these observations, a model to explain the results at the *SUP4* locus must also account for the following: inversions of the adjacent sequences often occur in concert with the deletions, and the *Xho*I site in delta 2 is often transferred to delta 4 but is never lost from delta 2.

To describe a mechanism for the deletions that we detected at the *SUP4* locus, we will consider the possibilities that the events can be initiated at different stages of the cell cycle. Previous researchers have argued that mitotic recombination occurs during either G1 or G2 (7, 8, 22, 40, 41, 54). We will describe two models—one in which the events initiate in G1 and another in which they initiate in G2.

In the G1 model for the 2.1-kb deletion (Fig. 5), the postulated gene conversion event is depicted as an intrastrand exchange. We propose that the pairing can occur as shown in Fig. 5A. Although there are several hypothetical ways to initiate a gene conversion event between the deltas in this configuration (14, 17, 30, 36, 52), we believe that the *RAD52* dependence of these events supports a double-strand-break-initiated conversion. Such an initiating break may occur anywhere within the *SUP4* delta-bounded region, followed by nuclease degradation to eventually expose deltas 4 and 5. The next step is the pairing and invasion of the homologous delta 1-2 region by the free delta 4 and delta 5 ends (Fig. 5B). As in the double-strand-break model (52), the 3′ ends act as the primers for repairing the breaks by using the delta 1-2 region as the template. The invasion and subsequent repair results in two Holliday structures flanking the repaired region. We next assume that resolution of the two Holliday structures in the same mode (i.e., across the inside strands or across the outside strands) results in the class I and II deletions. Resolution of the two Holliday structures in different modes results in a reciprocal exchange of the adjacent interval, generating the class III and IV deletion-inversions. The difference between classes I and III versus classes II and IV is the presence of the *Xho*I site in recombinant delta 2-4. The duplicated *Xho*I site results from a coconversion of the *Xho*I site from delta 2 during the conversion of the delta 1-2 junction. This coconversion may have arisen in two ways. It could have occurred by the degradation of delta 4 DNA past the position of the *Xho*I site (nucleotide 240) before it invaded and was subsequently repaired by using the delta 2 sequence as the template. Alternatively, the invading delta 4 DNA may have formed a heteroduplex with delta 2 that included the *Xho*I site. Next,

mismatch repair occurred, creating a homoduplex *Xho*I site in the delta 2-4 recombinant.

A G1 model can also be formulated for the 2.8-kb deletions. Two of the three 2.8-kb deletion classes (V and VI) appear to be the result of crossing-over between delta 3 and delta 5. However, the dependence on *RAD52* suggests that a double-strand break may also be involved in generating these deletions. Figure 5C shows a potential intermediate in a double-strand-break-initiated G1 event that would generate a 2.8-kb deletion. This intermediate is similar to one proposed in a model for the repair of the ends of a linear molecule during DNA transformation in yeast (46), an event that is sensitive to *rad52*. As shown in Fig. 5C, the initiating break occurs near delta 3, and the free end pairs with and invades delta 5. The break may just as likely occur in delta 5, with delta 3 used as the template for repair. In either case, a crossover results after the recombination complex is resolved. Deletions that retain or lose the *Xho*I site (class V and VI, respectively) are generated, depending on the position of the crossover junction. Class VII deletions, which contain an inversion, cannot be generated by simple reciprocal exchanges in G1, since in the sequenced class VII

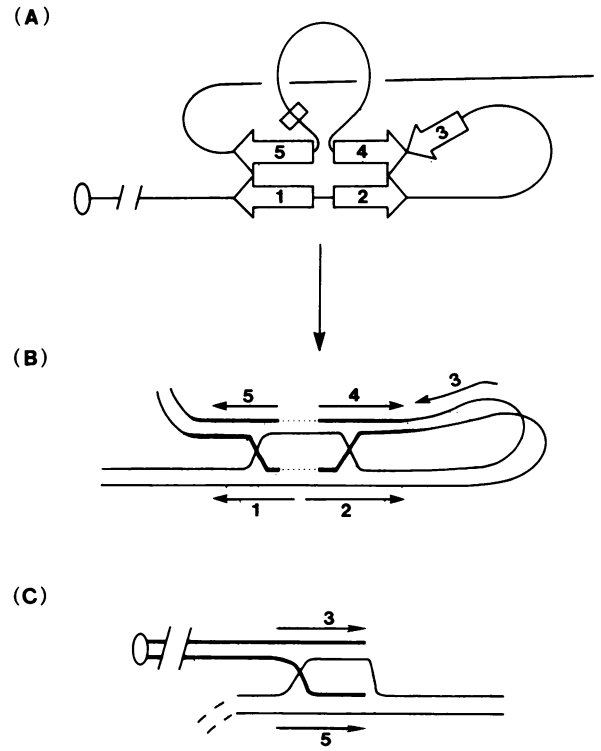


FIG. 5. G1 models for the *SUP4* rearrangements. (A) Postulated intrastrand pairing configuration for generating the 2.1-kb class I through IV deletions. (B) Double-strand-break repair intermediate for the structure in panel A. The thickened lines distinguish the invading broken ends of deltas 4 and 5 from the delta 1-2 template region. The dotted strands signify DNA repair synthesis resulting in gene conversion with the delta 1-2 junction as the template. The two Holliday junctions that flank the converted segment can be resolved, leading to crossover and noncrossover products. See text for details. (C) Double-strand-break intermediate for generating the 2.8-kb class V and VI deletions. The thickened lines indicate the delta 3 DNA adjacent to the broken end that paired and invaded the delta 5 region. The dashed DNA strands are the degraded *SUP4* region. Resolution of the Holliday junction as well as the displaced strand leads to rescue of a chromosome carrying the *SUP4* deletion.

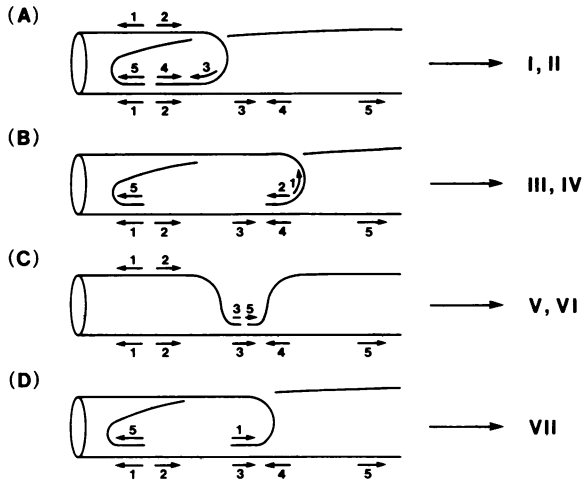


FIG. 6. G2 model for the *SUP4* rearrangements. In this figure, sister chromatids are shown attached to their centromere. The gap is shown on one of the chromatids and is repaired by using homologous, but not identical, sequences from the other. The gap repair occurs as shown in Fig. 5B. The pairing arrangement in panel A generates either a class I or class II deletion depending on the size of the gene converted segment. (B) Pairing that results in class III and class IV deletions. (C) One of two possible pairing arrangements that result in class V and class VI deletions. The other is the invasion of delta 5 with the broken ends of deltas 3 and 5. (D) Pairing arrangement that generates a class VII deletion.

deletion-inversion J69, the recombinant 1-3 delta contained at least 189 nucleotides from the 3′ end of delta 1, and the delta 1-5 recombinant contains at least 190 nucleotides of the 5′ end of delta 1. Thus, there are 46 duplicated delta 1 nucleotides in this rearrangement. A gene conversion event similar to the class I through IV 2.1-kb deletions shown in Fig. 5A can be proposed to explain the class VII events. In this model, for example, deltas 3 and 5 pair with and invade delta 1, repair by using delta 1 as the template, and finally resolve with a crossover. The model predicts that delta 1 sequences will occasionally contribute to the middle of the recombinant delta when resolution of the gene conversion does not generate an inversion. Since no delta 1 sequences were found in the two delta 3-5 recombinants that were sequenced, it is unlikely that such a conversion event generated the class V or VI deletions. Thus, the various 2.8-kb rearrangements observed cannot all be easily accommodated within one simple G1 model.

We will next consider a G2 model that involves sister chromatid exchange. Following a double-strand break on one chromatid, we postulate that the free ends invade the sister chromatid, as shown in Fig. 6. This leads to two Holliday structures flanking the converted region. For the structures shown in Fig. 6A, B, and D, only resolutions of both Holliday structures in the same mode lead to the observed conversion events, since resolutions that yield a crossover would result in a dicentric deletion chromosome and an acentric fragment, a lethal event in a haploid. Pairing of deltas 1 and 2 with deltas 5 and 4, respectively, and subsequent conversion leads to the 2.1-kb deletion (class I and II, Fig. 6A) and pairing of deltas 5 and 2 with deltas 1 and 4 leads to the deletion with an adjacent inversion (class III and IV, Fig. 6B). The coconversion of the *Xho*I site in class II and IV events is generated in the same way as described for the G1 model.

A 2.8-kb deletion is generated when a break occurs at or

near delta 3 or 5 and their free ends invade either delta 3 or 5 on the homologous sister chromatid (Fig. 6C). Depending on the position of the crossover, the *Xho*I site of delta 3 is either retained or lost. To generate the 2.8-kb deletion-inversion class (VII), we imagine that delta 5 and delta 1 pair and invade delta 1 and delta 3, respectively (Fig. 6D). This mechanism explains the duplication of delta 1 nucleotides found in the class VII deletion-inversion J69, since two delta 1 sequences contribute to the final recombinant.

We have presented two models for generating the deletions and inversions that we observed at the *SUP4* locus. The G1 model for classes I to IV predicts that the *Xho*I site of delta 2 could be lost due to mismatch correction of a proposed heteroduplex intermediate; however, this site was never lost in the 52 deletions that we examined. One possible explanation for this result is that the region of potential heteroduplex is short and that even when the site is included in it there is a mismatch correction bias to restore the *Xho*I site. This observation is more easily accommodated in the G2 model, in which the postulated lost *Xho*I site would occur in the delta 2 sequence of the sister cell that is not recovered after mitosis. Although we do not dismiss the G1 models for this reason, we favor the G2 model since it postulates one single mechanism that can generate all of the rearrangements that we observed. Interestingly, all of the deletion classes appear to be *RAD52* dependent, although the delta 3-5 direct-repeat recombinant classes are less so. These observations lend some support to the notion that the events occur by one single mechanism. This mechanism may also account for several deletions in yeast observed at the *CYC1* locus (25) and near *LEU2* and *HIS4* (39).

Finally, we propose that the models presented to explain the rearrangements at *SUP4* may be generally applicable to deletions observed in other eucaryotes. For example, *Alu-Alu* recombination events that delete the C-terminal domain of the human LDL receptor (16a; M. A. Lehrman, D. W. Russell, J. L. Goldstein, and M. S. Brown, personal communication) are analogous to the class V deletions that we have found at *SUP4*. In addition, a novel *Alu-Alu* recombinant has been found in which two inverted *Alu* sequences interact and result in a deletion (24). We propose that such rearrangements may occur by a template-mediated event as we have described for class I deletions. A similar mechanism may cause other *Alu*-associated exchanges, such as those seen at the globin loci (21, 33, 53) and on the Philadelphia chromosome (16).

In summary, we have shown that the rearrangements at the *SUP4* locus are most likely due to gene conversion events that occur between homologous repeated sequences within the region. Several models to explain these events have been proposed. Utilizing the powerful molecular genetic system of yeast, we are now in a position to find mutations in genes that affect this process in order to determine the precise rearrangement mechanism(s).

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