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Evolution of the Dispersed *SUC* Gene Family of *Saccharomyces* by Rearrangements of Chromosome Telomeres

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The *SUC* gene family of *Saccharomyces* contains six structural genes for invertase (*SUC1* through *SUC5* and *SUC7*) which are located on different chromosomes. Most yeast strains do not carry all six *SUC* genes and instead carry natural negative (*suc*^{0}) alleles at some or all *SUC* loci. We determined the physical structures of *SUC* and *suc*^{0} loci. Except for *SUC2*, which is an unusual member of the family, all of the *SUC* genes are located very close to telomeres and are flanked by homologous sequences. On the centromere-proximal side of the gene, the conserved region contains X sequences, which are sequences found adjacent to telomeres (C. S. M. Chan and B.-K. Tye, Cell 33:563–573, 1983). On the other side of the gene, the homology includes about 4 kilobases of flanking sequence and then extends into a Y′ element, which is an element often found distal to the X sequence at telomeres (Chan and Tye, Cell 33:563–573, 1983). Thus, these *SUC* genes and flanking sequences are embedded in telomere-adjacent sequences. Chromosomes carrying *suc*^{0} alleles (except *suc2*^{0}) lack *SUC* structural genes and portions of the conserved flanking sequences. The results indicate that the dispersal of *SUC* genes to different chromosomes occurred by rearrangements of chromosome telomeres.

The *SUC* gene family of *Saccharomyces* includes six structural genes for the sucrose-hydrolyzing enzyme invertase: *SUC1* through *SUC5* and *SUC7*. Each *SUC* gene encodes both a secreted and an intracellular form of invertase. The genes of the family are dispersed throughout the yeast genome. *SUC* genes have been mapped genetically to loci on the following chromosomes: *SUC1*, chromosome VII (9); *SUC2*, IX; *SUC3*, II; and *SUC5*, IV (19). The *SUC4* and *SUC7* genes are located on chromosomes XIII and VIII, respectively (J. Celenza and M. Carlson, unpublished data), but their positions have not been mapped. An unusual feature of this gene family is that closely related *Saccharomyces* strains often differ in *SUC* genotype. Most strains do not carry *SUC*$^+$ alleles at all six loci and carry natural negative alleles, designated *suc*^{0} alleles, at some or all *SUC* loci. Preliminary physical analysis of the different *SUC* and *suc*^{0} alleles has shown that all active *SUC* genes are homologous to the cloned *SUC2* gene (4, 5). The *suc2*^{0} allele in many strains is a mutant gene or pseudogene; it produces two mRNAs of the expected sizes with normal regulation but does not encode active invertase (3). Genetic studies have shown that *suc2*^{0} can mutate to an active Suc$^+$ state and can recombine with *suc2* amber alleles to generate an active *SUC2* gene (7). Other *SUC* loci bearing *suc*^{0} alleles, however, lack *SUC* DNA sequences (4). The frequent absence of *SUC* gene infomation at known *SUC* loci suggested that *SUC* genes have moved to different chromosomal locations since the divergence of closely related *Saccharomyces* strains.

To investigate the mechanism by which these genomic rearrangements have occurred, we determined the physical structures of *SUC* loci carrying both *SUC* and *suc*^{0} alleles by molecular cloning and blot hybridization analysis. We found that all of the *SUC* genes except *SUC2* are located very close to chromosome telomeres, and our results suggest that the evolution of the *SUC* gene family involved rearrangements of chromosome termini.

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MATERIALS AND METHODS

Strains and genetic methods. Yeast strains and genotypes are listed in Table 1. Strains R251-4A, 1412-4D, SS-12A, 2080-8C, DBY615, and DBY782 were used for all blot hybridization analyses of *SUC* loci except where otherwise stated. Standard yeast genetic procedures for crossing, sporulation, and tetrad analysis were followed (28). Media and methods for scoring for ability to ferment sucrose have been described previously (6).

Preparation and analysis of DNA. Plasmid DNAs and yeast DNAs were prepared as described previously (26). Phage DNAs were prepared as described by Davis et al. (12). Large-scale preparation of phages with purification on CsCl gradients was carried out by the procedures of Maniatis et al. (16). Restriction digests, gel electrophoresis, recovery of DNA from agarose gels, subcloning, and bacterial transformation were carried out as described previously (8, 26). Hybridization was carried out at 42°C in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–50 mM sodium phosphate (pH 7)–1× Denhardt solution–0.1% sodium dodecyl sulfate–100 μg of denatured sonicated salmon sperm DNA per ml. Filters were washed at 50°C in 0.1× SSC–0.1% sodium dodecyl sulfate. Radioactively labeled probes were prepared by nick translation (23).

Isolation of cloned *SUC* DNA. A clone containing the 8-kilobase (kb) *Eco*RI fragment on which the *SUC7* gene is located was recovered from a library of *Eco*RI fragments of DBY673 (*SUC7*) DNA inserted into λgt7 (25). The library was screened by plaque hybridization (1) for sequences homologous to the *SUC2* structural gene probe pRB117 (3). A library of cloned genomic DNA from strain DBY615 (*SUC7*) was constructed by digesting the DNA with *Bam*HI, ligating the resulting fragments to *Bam*HI-cleaved DNA of phage vector Charon 30 (24), and packaging the ligation mixture into phage coats (16). A phage homologous to the 0.9-kb *Eco*RI-*Bam*HI fragment containing the 5′ portion of the *SUC7* structural gene was identified by plaque hybridization. The restriction map of this clone was identical to that of genomic DNA from DBY615, and other *SUC7*

TABLE 1. List of yeast strains

Strain	Genotype	Source
S288C	*MATα SUC2 gal2*	G. Fink
R251–4A	*MATα SUC1 ura1 ade2*	Yeast Genetic Stock Center
1412–4D	*MATa SUC3 MAL3 MEL1 MGL2 MGL3 ade2*	Yeast Genetic Stock Center
SS-12A	*MATα SUC4 his4*	Yeast Genetic Stock Center
2080–8C	*MATa SUC5 ade6*	Yeast Genetic Stock Center
DBY615	*MATa SUC7 ura3–34*	F. Lacroute
DBY673	*MATa SUC7 ura3–3*	F. Lacroute
DBY782[a]	MATα SUC2 ade2 gal2	D. Botstein
DBY939[a]	*MATα suc2–215 ade2 gal2*	M. Carlson
MCY87	*MATα SUC3 MAL3 ade2 his4*	This work
MCY526	*MATα SUC7 his4 lys2 ura3*	This work
MCY619[a]	*MATa suc2–215 ura3 his4 gal2*	This work
MCY908	*MATα SUC4 ura3*	This work

[a] Strain is isogenic or congenic to S288C.

strains, throughout the 3.5-kb region upstream from the gene but was not identical farther upstream, indicating that this phage carries sequences that are not found at the genomic *SUC7* locus. We therefore cloned this upstream region by a different method. The 2.5-kb *Hin*dIII-*Eco*RI fragment upstream from the *SUC7* gene was subcloned into the integrative plasmid vector YIp5 (2), and the resulting plasmid, pLW11-4, was inserted into the *SUC7* locus of MCY526 by targeted transformation (15, 22). The plasmid and adjacent sequences extending upstream to the next *Bam*HI site were recovered by digesting genomic DNA from the transformed strain with *Bam*HI, ligating, and transforming bacteria with selection for ampicillin resistance.

A library of cloned genomic DNA from strain R251-4A (*SUC1*) was prepared by ligating *Bgl*II fragments of R251-4A DNA to purified Charon 30 phage arms and packaging the ligated mixture. Clones containing DNA from the *SUC1* locus were identified by plaque hybridization using as probes two subcloned restriction fragments from the cloned *SUC7* locus (probes j and l, Fig. 1).

To recover cloned DNA from the *SUC4* locus, we subcloned the *Eco*RI-*Bam*HI fragment containing the 5′ half of the *SUC7* gene into YIp5 and integrated this plasmid, pLW15-8, at the *SUC4* locus of MCY908 by transformation; these *Eco*RI and *Bam*HI sites are conserved at *SUC4*. The plasmid and adjacent sequences from the *SUC4* locus were recovered by partially digesting genomic DNA from the yeast transformant with *Bgl*II, ligating, and transforming bacteria to ampicillin resistance. The resulting clones carry hybrid *SUC4-SUC7* sequences in the region between the *Eco*RI and *Bam*HI sites; all sites shown in Fig. 1 are present at *SUC4*.

Additional clones of the *SUC2* locus were recovered by screening libraries prepared by N. Neff for phages homologous to either of the two *Hin*dIII fragments containing the left and right ends of the *SUC2* DNA cloned in pRB54 (3; Fig. 1). The libraries were constructed by partially digesting DNA from strains isogenic to S288C with *Sau*3AI and inserting the fragments into λBF101 (21).

Preparation of restriction maps of *SUC3* and *SUC5* loci. Restriction maps of all six *SUC* loci were generated by blot hybridization analysis of genomic DNA fragments from strains carrying one *SUC* gene. Restriction fragments derived from each *SUC* locus were identified by their homology to the *SUC2* structural gene or to unique sequence probes from the cloned *SUC7* locus (probes j, k, and l, Fig. 1). The conservation of restriction sites among the *SUC3*, *SUC4*, *SUC5*, and *SUC7* loci enabled us to deduce maps of *SUC3* and *SUC5*. This analysis also confirmed that the cloned regions corresponded faithfully to the chromosomal loci.

Isolation of cloned *suc*^{0} DNA. Cloned DNA from each *suc*^{0} locus was recovered from a library constructed by partial digestion of DBY939 (*suc2-215*) genomic DNA with *Sau*3AI and insertion of the resulting fragments into the *Bam*HI site of plasmid vector YEp24 (3). The library was screened by colony hybridization (14) using probes specified in Results. For each *suc*^{0} locus, multiple overlapping clones were isolated. Restriction sites were mapped in all clones to generate a map of the locus.

Identification of *suc1*^{0} and *suc3*^{0} clones. The *suc1*^{0} and *suc3*^{0} clones were initially identified by analysis of the *MAL* sequences on these clones (see Fig. 5). These clones were derived from the maltose-nonfermenting strain DBY939, which does not carry *MAL1* or *MAL3*. However, yeast strains commonly carry the cryptic *MAL1g* and *MAL3g* alleles (17, 18, 20), and both the *suc1*^{0} and *suc3*^{0} clones were found to complement a *MAL1p* tester strain, indicating that they confer *MALg* function (M. Charron and C. Michels, personal communication). The clones were initially designated *suc1*^{0} and *suc3*^{0} based on the size of the *Hin*dIII fragment homologous to cloned *MAL6* DNA (see Fig. 5). The *suc1*^{0} clone gives rise to a 10.7-kb *Hin*dIII fragment that is characteristically associated with the *MAL1g* locus (17, 18, 20), and the 8.1-kb *Hin*dIII fragment from *suc3*^{0} is the same size as fragments from other MAL3g loci (18; M. Charron and C. Michels, personal communication). To confirm these assignments, genetic analysis was carried out as follows. A restriction fragment length polymorphism distinguishing the *SUC1*, *SUC3*, *suc1*^{0}, and *suc3*^{0} loci was identified; digestion with *Xho*I generated from each locus a fragment of a different size that was homologous to probe a from the *SUC1* locus. The 9- and 20-kb fragments derived from the *suc1*^{0} and *suc3*^{0} loci of DBY939 were assigned based on the known distance between *Xho*I sites in the cloned *suc1*^{0} DNA (see Fig. 5). Tetrad analysis of a cross of MCY619 (*suc1*^{0} *suc2*$^-$ *suc3*^{0}; isogenic to DBY939) to the *SUC1* strain R251-4A provided confirming evidence that the cloned DNA designated *suc1*^{0} is linked to *SUC1*; in the two tetrads examined, the 9-kb *suc1*^{0} fragment segregated 2:2 from the *SUC1* gene and from the *Xho*I fragment associated with *SUC1*. To obtain further evidence that the cloned *suc3*^{0} DNA is linked to *SUC3*, MCY619 was crossed to MCY87 (*suc1*^{0} *suc2*^{0} *SUC3*), which carried the 9-kb *suc1*^{0} fragment and a >25-kb fragment derived from the *SUC3* locus. Analysis of two tetrads showed that the 20-kb *suc3*^{0} fragment segregated 2:2 from the *SUC3* gene and from the >25-kb fragment; each spore carried the 9-kb *suc1*^{0} fragment (data not shown). These results supported the other evidence for the identification of the cloned *suc3*^{0} DNA.

RESULTS

Regions flanking the *SUC2* gene are not conserved at other *SUC* loci. Because all the *Saccharomyces* strains tested appeared to carry *SUC* gene information at the *SUC2* locus (either the *SUC2* gene or the defective *suc2*^{0} allele; 4), it seemed likely that *SUC2* might be the progenitor of the *SUC* gene family. We therefore began our study by examining the homology between the *SUC2* locus and other *SUC* loci. The *SUC2* locus, including 7.5 kb of flanking sequence 5′ to the

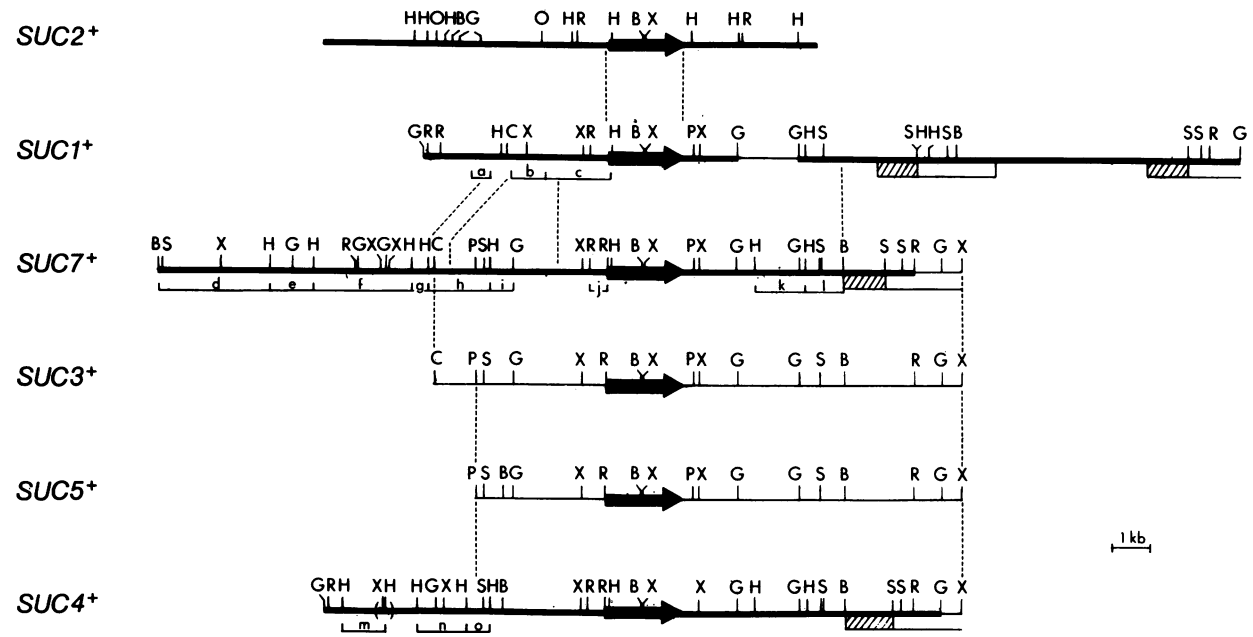

FIG. 1. Structures of the *SUC* loci. Heavy lines indicate cloned DNA; thin lines indicate regions mapped by blot hybridization analysis, where not all sites are known. The *SUC* structural genes are represented by arrows pointing in the direction of transcription. Hatched bars represent 131 elements. Open bars indicate homology to Y sequence. Dashed lines indicate regions of homology between different loci; the boundaries of some regions have not been determined precisely (see text). The homology of Y′ sequences at *SUC1* to other Y′ sequences is not indicated. Restriction fragments used as probes are labeled a to o. The map of the *SUC2* locus is a map of pRB54 (3). Restriction sites: B, *Bam*HI; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sal*I; C, *Sac*I; X, *Xba*I; O, *Xho*I. Only those *Pst*I sites identified by blot hybridization are shown, and not all *Sac*I sites are shown.

structural gene and 3.7 kb of 3′ flanking sequence, had been cloned previously from DBY939, a strain isogenic to the common laboratory strain S288C and carrying a *suc2* amber allele (3). To determine if the sequences flanking the *SUC2* gene were conserved at other *SUC* loci, restriction fragments spanning the entire cloned region were radioactively labeled and used in blot hybridization experiments (29) to probe genomic DNA from six strains, each carrying one of the six active *SUC* genes (*SUC1* through *SUC5* and *SUC7*) (Fig. 1). As expected from previous studies (4), probes containing *SUC2* structural gene sequences hybridized to the $suc2^0$ mutant gene and to each of the other *SUC* structural genes (data not shown). In contrast, probes containing *SUC2* flanking sequences displayed no homology to sequences at the *SUC1*, *SUC3*, *SUC4*, *SUC5*, and *SUC7* loci but hybridized only to fragments derived from the $suc2^0$ locus of each of the other strains; Fig. 2 shows the hybridization patterns obtained with two such probes. These results indicated that only the structural gene, and perhaps close flanking sequences, are conserved between *SUC2* and other *SUC* loci.

Extensive homology among the *SUC1*, *SUC3*, *SUC4*, *SUC5*, and *SUC7* loci. Previous studies showed that large restriction fragments of identical size are generated from the *SUC1*, *SUC3*, *SUC4*, *SUC5*, and *SUC7* loci by digestion with several restriction enzymes (4, 5). These findings suggested that these *SUC* genes are flanked by homologous sequences in which restriction sites have been conserved. To examine this possibility, we cloned the *SUC1*, *SUC4*, and *SUC7* genes, including in each case 15 to 20 kb of flanking sequence (see Materials and Methods). The restriction maps of these cloned regions are shown in Fig. 1. Restriction maps of the remaining two loci, *SUC3* and *SUC5*, were generated by blot hybridization analysis (29) of genomic DNA fragments, as described in Materials and Methods. The homology among the different loci was assessed by the conservation of both restriction sites and homologous sequences.

The *SUC3*, *SUC4*, *SUC5*, and *SUC7* loci initially appeared to be more closely related to one another than to *SUC1*, and therefore the relationships of these four will be considered first. The conservation of restriction sites and unique sequences (probes j, k, and l in Fig. 1) indicated that a region of homology at least 13 kb in size is common to the *SUC3*, *SUC4*, *SUC5*, and *SUC7* loci. On the 3′ side of the *SUC* structural genes, the homology extends beyond the mapped region. On the 5′ side of the *SUC* genes, the extent of the homology appeared to vary, depending on the pair of loci being compared. The *SUC3* and *SUC7* loci appeared to share a larger region of homology with one another than with *SUC4*, as judged by the fact that the restriction maps of *SUC3* and *SUC7* are identical as far as 4.5 kb upstream from the 5′ end of the *SUC* structural gene whereas the maps of *SUC4* and *SUC7* diverge about 3.5 kb upstream from the *SUC* gene. We undertook experiments to characterize the 5′ portions of these homology regions more thoroughly, beginning with the *SUC4* locus. To locate the 5′ boundary of the region of homology between *SUC4* and the other loci, restriction fragments from the *SUC4* clone were used in blot hybridization experiments to probe both cloned DNAs and genomic DNAs from six strains, each carrying one *SUC* gene. Probes representing sequences lying farther than 3.5 kb upstream from the *SUC4* gene were found to contain unique sequences that are not present at other *SUC* loci (probes m and n in Fig. 1). A probe containing sequences located closer to the *SUC4* gene (probe o) hybridized to the cloned *SUC7* locus and was found also to be repeated many

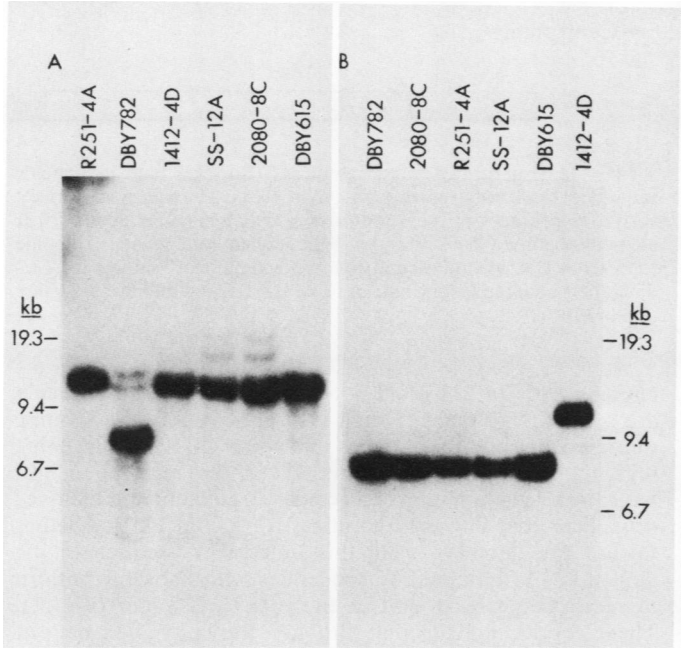

FIG. 2. *SUC2* flanking sequences are not homologous to other *SUC* loci. Genomic DNAs were digested with *Bam*HI (A) or *Eco*RI (B), and the resulting fragments were separated by electrophoresis in a 0.5% agarose gel. Fragments larger than 0.5 kb were transferred to nitrocellulose (29), and those homologous to ^{32}P-labeled probes were detected by hybridization and autoradiography. Probes were prepared from purified restriction fragments from cloned *SUC2* DNA: (A) 1.3-kb *Hin*dIII fragment located immediately 3′ to the *SUC2* gene; (B) 3.2-kb *Hin*dIII fragment located 5′ to *SUC2* (Fig. 1). In each sample, a fragment derived from the *SUC2* or *suc2*^{0} locus hybridized to the probe, but no homology to sequences at any other *SUC* locus was detected (see Fig. 1 for predicted sizes of such fragments). The *Bam*HI fragments shown in panel A have previously been shown to be derived from the *SUC2* and *suc2*^{0} loci (4, 5); faint bands are due to partial digestion products and impurity of the restriction fragment used as probe. The *Eco*RI fragment detected in *SUC2* DNA is the size expected from the maps of large *SUC2* DNA clones (maps not shown) and appears to be conserved at the *suc2*^{0} loci of all strains except 1412-4D. Relevant genotypes: R251-4A (*SUC1*), DBY782 (*SUC2*), 1412-4D (*SUC3*), SS-12A (*SUC4*), 2080-8C (*SUC5*), DBY615 (*SUC7*).

times in each yeast genome. Additional experiments confirmed that sequences lying between probe o and the *SUC4* gene are conserved at the *SUC7* locus (data not shown). Thus, these results indicated that the homology between *SUC4* and other *SUC* loci extends up to, but no farther than, 3.5 kb upstream from the *SUC4* gene.

A similar analysis of sequences from the *SUC7* locus showed that regions farther than 5 kb upstream from the gene contain unique sequences specific to the *SUC7* locus (probes d, e, and f in Fig. 1), and regions closer to the gene (probes g, h, and i) include repeated sequences. Thus, at the *SUC7* locus the homology to other loci can extend no farther than 5 kb from the *SUC7* gene. The conservation of a *Sac*I site 4.5 kb upstream from both *SUC3* and *SUC7* suggests that the homology between these two loci extends to this site, and analysis of the cloned *suc3*^{0} locus, presented below, supported this conclusion.

We next examined the homology between *SUC1* and the other *SUC* loci, beginning with the homology on the 5′ side of the *SUC* gene. Restriction fragments from the cloned *SUC1* DNA were tested for homology to the cloned *SUC7* DNA and to all six genomic *SUC* loci by blot hybridization analysis. The homology to *SUC4*, *SUC5*, and *SUC7* was shown to extend no farther than 3 kb upstream from the *SUC1* gene; a 0.5-kb sequence located just beyond 3 kb upstream from the *SUC1* gene (probe a in Fig. 1) did not hybridize to these loci. This sequence did, however, hybridize to a locus besides *SUC1* or *suc1*^{0} in all strains examined, and we suspected that this second locus was *SUC3* or *suc3*^{0} because of the following data: *SUC1* and *MAL1* are tightly linked genetically, and *SUC3* and *MAL3* are tightly linked (19); yeast strains often carry *MAL* information at the *MAL1* and *MAL3* loci regardless of their ability to ferment maltose (11, 17, 18, 20); and, finally, the restriction map of the *SUC1* clone is identical to that of cloned *MAL6* DNA in the region from which probe a was derived (20; R. Needleman and C. Michels, personal communication; see Fig. 5). Evidence that the *SUC1* clone contains *MAL* sequences and that the second locus detected is linked to *SUC3* is presented below and in Materials and Methods. The relationship between *SUC1* and *SUC3* is therefore a special case: the region of homology appears to extend from the *SUC* locus into the neighboring *MAL* locus. Analysis of cloned *suc1*^{0} and *suc3*^{0} DNAs (see below) was consistent with this interpretation.

Further study of the homology between *SUC1* and the other loci within the 5′ flanking region was hindered by the finding that restriction fragments closer to the *SUC1* gene than 3 kb contain repeated DNA (probes b and c) and were therefore not useful in assessing homology to other *SUC* loci by blot hybridization analysis of genomic DNAs. Comparison of the restriction maps of the cloned *SUC1* and *SUC7* DNAs, however, revealed that both clones contain a conserved series of restriction sites near the *Sac*I site located 2.7 kb from the *SUC1* gene and 4.5 kb from the *SUC7* gene; in both cases the *Sac*I site is closely flanked by sites for *Hin*dIII (Fig. 1), *Nco*I, and *Eco*RV (not shown) in equivalent positions. Analysis of the homology between restriction fragments from the cloned *SUC1* and *SUC7* DNAs indicated that the two clones also contain homologous sequences extending at least 1 kb upstream from the *SUC* genes but that in the intervening region the *SUC7* clone contains sequences absent from *SUC1* (Fig. 1). These data suggest that the homology between *SUC1* and *SUC7* extends in the 5′ direction from the *SUC* genes to the *Sac*I sites but is interrupted by a substitution at *SUC1*.

In the 3′ direction from the *SUC1* gene, the homology to *SUC3* through *SUC7* extends for 4 kb, as judged by conservation of restriction sites and homologous unique sequences (probes k and l). Beyond that point, all the cloned DNAs contain repeated sequences. Studies described in the next section, however, showed that despite the divergence of the restriction maps, the homology extends farther.

In summary, analysis of the homology between *SUC1* and other loci was complicated by the presence of a substitution in the 5′ conserved sequence flanking the *SUC1* gene. If this difference is ignored, the relationships among the loci can be summarized as follows. The *SUC1* and *SUC3* loci share the largest region of 5′ homologous sequence, which extends beyond the mapped region of *SUC3* shown in Fig. 1 into the neighboring *MAL* locus. The region of homology between the *SUC7* locus and *SUC1* and *SUC3* extends about 5 kb upstream from the *SUC7* gene. Although no direct analysis of *SUC5* has been carried out, the failure of unique probes from the other loci to hybridize to *SUC5* implies that the 5′ boundary of the homology to *SUC1*, *SUC3*, and *SUC7* lies no farther than 5 kb from the gene. The region of homology

between *SUC4* and all of the others extends only about 3.5 kb from the gene.

Cloned *SUC1*, *SUC4*, and *SUC7* DNAs contain sequences found adjacent to telomeres. The possibility that some or all of the *SUC* loci are located close to telomeres was suggested by two observations. First, the *SUC2*, *SUC3*, and *SUC5* loci have been mapped genetically, and each is the most centromere-distal marker on its chromosome arm (19). Our recent genetic data show that *SUC1* is the most distal marker on the right arm of chromosome VII (9). Second, we found that the regions flanking the *SUC1* and *SUC7* structural genes contain an unusually large number of repetitive sequences relative to a typical cloned yeast DNA segment (Fig. 1 and data not shown), and regions adjacent to telomeres are known to contain repeated sequences, called X and Y′ sequences (10, 30). The X elements are a heterogeneous family of sequences located centromere proximal to the poly($C_{1-3}A$) tracts (27) at telomeres (Fig. 3). The 6.7-kb Y′ sequence is composed of two elements, designated 131 and Y. Telomeres carry zero to four copies of the Y′ sequence located between the X sequence and the end of the chromosome (30). We therefore tested the cloned *SUC1*, *SUC4*, and *SUC7* DNAs for homology to the X and Y′ sequences by blot hybridization analysis. Probes were prepared from plasmid 131B, which carries the Y′ sequence, and from plasmid 131A, which carries a 131 element, X sequence, and adjacent centromere-proximal regions (10).

The cloned *SUC1*, *SUC4*, and *SUC7* DNAs were found to contain sequences homologous to the Y′ sequence at positions 3′ to the *SUC* structural genes (Fig. 1). In each case, the Y′ sequence is oriented such that the 131 element is positioned closer to the *SUC* gene than is the Y segment. The Y′ sequences at telomeres are known to be oriented such that the 131 element is centromere proximal to the Y portion (10, 30). These results suggested that telomeres are located 3′ to these *SUC* genes. The 131 elements are located 4 kb distal to the *SUC4* and *SUC7* genes and presumably are present at the corresponding positions relative to the *SUC3* and *SUC5* genes because restriction sites in this region are conserved. A 131 element is located about 5 kb distal to *SUC1*. The additional sequence that displaces the first 131 element 1 kb farther from the *SUC1* gene than is the case at other loci has not been characterized except to show that it is not homologous to the Y sequence or to the X sequence in plasmid 131A.

Hybridization to plasmid 131A showed that the cloned *SUC4* and *SUC7* DNAs contain sequences homologous to the X sequence within the conserved regions 5′ to the *SUC* genes (see Fig. 4); the *SUC3* and *SUC5* loci presumably carry X sequences at analogous positions. The cloned *SUC1* DNA also showed homology to plasmid 131A in regions 5′ to the *SUC1* gene; however, the *SUC1* DNA shares sequences besides X with 131A (see below) and appears to carry little if any X sequence. The presence of X sequences 5′ to the *SUC* genes, and therefore centromere proximal to the Y′ elements distal to *SUC* genes, is consistent with the idea that telomeres are located 3′ to the *SUC* genes. These *SUC* genes and their immediate flanking sequences thus appear to be embedded in telomere-adjacent sequences.

The Y′ sequences at the *SUC* loci were compared with previously cloned Y′ sequences. The Y′ elements at the *SUC4* and *SUC7* loci have restriction maps identical to that of the 5.2-kb variant in plasmid 131S isolated by Chan and Tye (10). The *SUC1* locus, however, appears to carry a novel variant Y′ sequence. The cloned *SUC1* DNA contains two 131 elements, each with the *Nco*I, *Cla*I, and *Sal*I sites

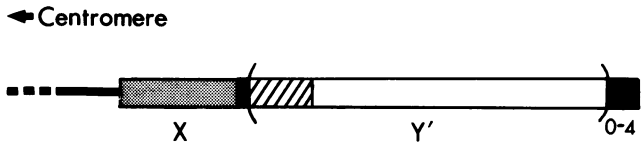

FIG. 3. Structure of yeast telomeres. Stippled bars indicate X sequence. Dark bars represent $C_{1-3}A$ repeats. Telomeres may carry zero to four copies of the Y′ sequence, which is composed of a 131 element (hatched bar) and the Y sequence (open bar). The line represents DNA located centromere proximal to X sequence. This diagram is adapted from Chan and Tye (10) as modified by Walmsley et al. (30).

characteristic of 131 (data not shown). These 131 elements are spaced 7 kb apart, which is approximately the spacing expected for a Y′ sequence, and parts of the intervening region are homologous to Y; however, this region does not have the restriction map of a typical Y′ element and includes sequences that did not hybridize to plasmid 131B. Farther toward the telomere from this unusual Y′ sequence, the cloned DNA contained Y′ sequences with the same pattern of restriction sites found at the other *SUC* loci (Fig. 1). These results indicate that although the restriction map of the *SUC1* locus diverges from that of the other *SUC* loci downstream from the gene, the *SUC1* locus carries telomere-adjacent sequences in an approximately equivalent position.

The *SUC2* locus is the distal marker on chromosome IX, but no repeated sequences were found within the cloned DNA analyzed above (Fig. 1). To investigate the possibility that *SUC2* also is near a telomere, additional clones extending 18 kb in the 5′ direction and 14 kb in the 3′ direction were isolated (see Materials and Methods). No homology to plasmid 131A was detected (data not shown). Thus, the *SUC2* gene is an unusual member of the family with respect to both its flanking sequences and its position relative to the telomere.

Structures of the *suc*^{0} loci. The structures of the *SUC1*, *SUC3*, *SUC4*, *SUC5*, and *SUC7* loci suggested that *SUC* genes have been dispersed to different chromosomes by rearrangments of chromosome termini. It was consequently of interest to determine the structure of these chromosome termini when a *SUC* gene is not present, that is, when the chromosome bears a *suc*^{0} allele. We therefore cloned the *suc1*^{0}, *suc3*^{0}, *suc4*^{0}, and *suc7*^{0} alleles.

Clones carrying the *suc*^{0} alleles were isolated from a library (3) of cloned DNA of DBY939, a strain that carries *suc*^{0} alleles at all loci except *SUC2*, as described above. Clones of the *suc4*^{0} or *suc7*^{0} locus were identified by hybridization to probes representing unique sequences located upstream from the 5′ boundary of the homology region at the *SUC4* or *SUC7* locus (probes f and n, Fig. 1). Clones carrying *suc1*^{0} and *suc3*^{0} were recovered with probe a from the cloned *SUC1* DNA, which was homologous to both loci. The evidence for the identities of these two clones is described in Materials and Methods.

The cloned *suc1*^{0}, *suc4*^{0}, and *suc7*^{0} DNAs were tested for homology to the 131, X, and Y sequences. The hybridization patterns indicated that each clone contains a 131 element and part of a Y sequence, and the restriction maps confirmed the presence of sites characteristic of 131 and Y (Fig. 4). The Y′ element in the *suc1*^{0} clone resembles the Y′ element in plasmid 131B. The *suc4*^{0} and *suc7*^{0} clones each contain a Y′ element similar to the variant found in plasmid 131S (10).

We were surprised to observe that much of the cloned

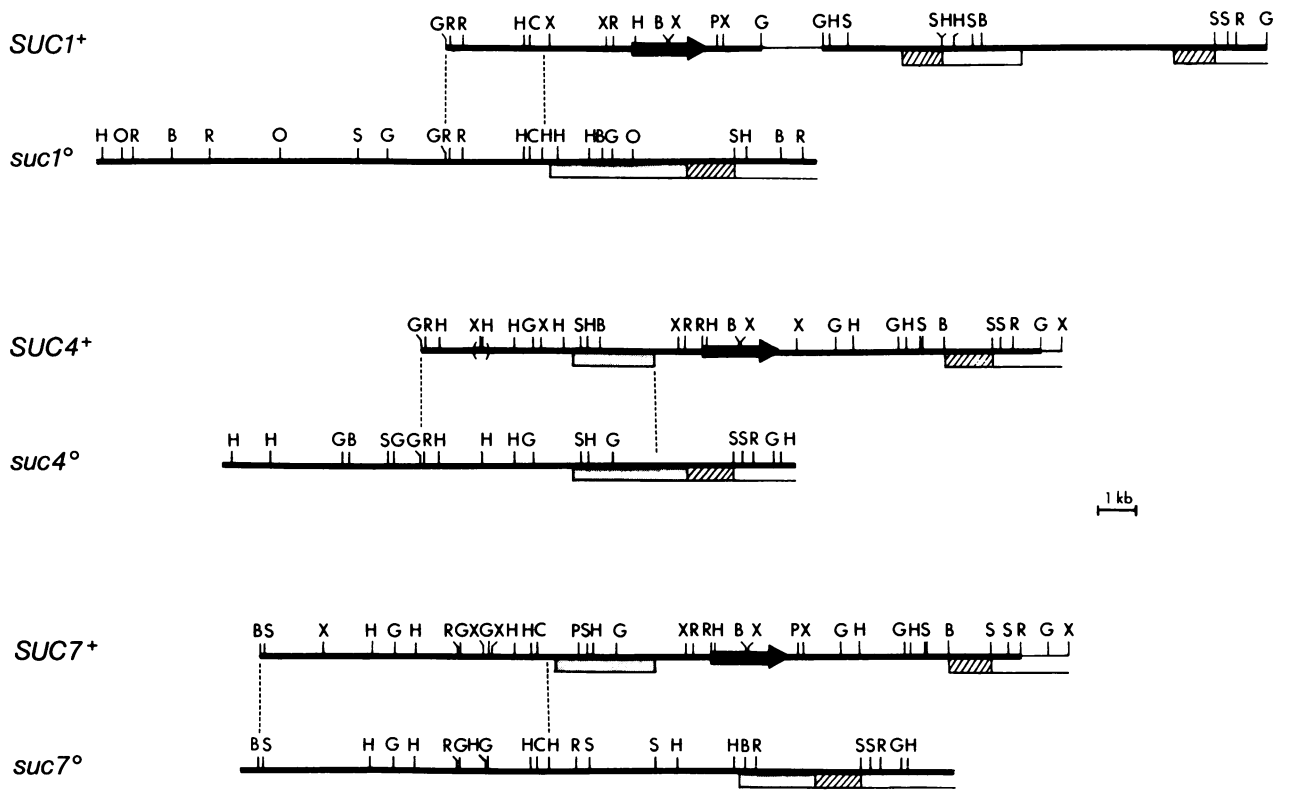

FIG. 4. Comparison of *SUC* and *suc*^{0} loci. Symbols and abbreviations are as in Fig. 1. Dashed lines indicate regions of homology between corresponding *SUC*$^+$ and *suc*^{0} loci; Y′ elements are also homologous. Stippled bars indicate regions of X sequence; $C_{1-3}A$ repeats have not been identified. The X sequences of *suc1*^{0} and *suc7*^{0} were identified by Chan and Tye (10) in their clones 131A and 131N, which carry *Sal*I fragments included within the cloned *suc1*^{0} and *suc7*^{0} DNAs, respectively. Both *suc1*^{0} and *suc7*^{0} share with 131A and 131N additional sites not included on these maps; the only discrepancy is that we have mapped two adjacent, approximately 0.5-kb *Hin*dIII fragments in *suc1*^{0} whereas only one of these was identified in 131A. The location of X sequences at the *suc4*^{0}, *SUC4*, and *SUC7* loci was determined on the basis of homology between these loci and *suc1*^{0} (Fig. 1 and 5); the position at which X sequence begins 5′ to the *SUC4* and *SUC7* genes has not been determined exactly. Each of the 131 elements in the *suc*^{0} clones includes the expected *Cla*I site very close to the *Sal*I site (not shown). Not all *Sac*I or *Pst*I sites are shown, and *Xba*I sites have not been mapped in the *suc*^{0} clones.

suc1^0 DNA hybridized to plasmid 131A. Inspection of the restriction maps revealed that our cloned DNA included the 10-kb *Sal*I DNA fragment present in 131A; the comigration of many restriction fragments during gel electrophoresis confirmed this conclusion. Similarly, the cloned *suc7*^{0} DNA appeared to contain the *Sal*I fragment present in plasmid 131N of Chan and Tye (10). Our cloned DNAs are most likely identical to those of Chan and Tye because all these clones were derived from DNA of strains isogenic to S288C. The regions identified as X sequence by Chan and Tye are indicated on the maps in Fig. 4 and 5.

Comparison of *SUC* and *suc*^{0} loci. The restriction maps of the cloned *suc*^{0} loci and the corresponding *SUC* loci are compared in Fig. 4. In each case the map of the *suc*^{0} DNA is nearly identical to that of the *SUC* DNA throughout the unique sequences located 5′ to the region conserved among *SUC* loci, and the homology extends partway into the conserved 5′ flanking region. The amount of this conserved flanking sequence that is present at the *suc*^{0} locus varies among the different loci. As expected, the *suc*^{0} loci lack the *SUC* gene and portions of the conserved flanking sequence. Formally, the relationships between *suc1*^{0} and *SUC1* and between *suc7*^{0} and *SUC7* appear to be substitutions of the region centromere proximal to the Y′ element. In addition, *suc1*^{0} carries a common Y′ sequence rather than the unusual variant present distal to the *SUC1* gene. The *SUC4* locus appears to be related to *suc4*^{0} by the insertion of an approximately 7-kb segment containing the *SUC* gene or by the substitution of a 7-kb segment for a portion of the X sequence at *suc4*^{0}. The data do not allow us to distinguish between these possibilities because the endpoint of the homology between *SUC4* and *suc4*^{0} within the X sequence has not been precisely determined.

The relationships of the cloned *suc1*^{0}, *suc3*^{0}, *suc4*^{0}, and *suc7*^{0} DNAs to one another are indicated in Fig. 5. Each of the *suc*^{0} clones has some homology to other *suc*^{0} clones in the region 5′ to the point of divergence between the *suc*^{0} locus and the corresponding *SUC* locus. In the case of *suc1*^{0} and *suc3*^{0}, the homology is extensive. The restriction maps of the two loci are similar throughout a 7-kb region, which contains eight conserved restriction sites in addition to those shown, and hybridization analysis confirmed the presence of homologous sequences. The two maps are also very similar to the map of cloned *MAL6* DNA (20) in the 3.5-kb region indicated (Fig. 5), and both the *suc1*^{0} and *suc3*^{0} clones hybridize to *MAL6* DNA probes within this region (M. Charron and C. Michels, personal communication). The homology between the *suc4*^{0} locus and the *suc1*^{0} and *suc3*^{0} loci extends from the boundary of the unique sequences specific to *suc4*^{0} through a region of X sequence and into the adjoining Y′ element. The homology between *suc7*^{0} and the other *suc*^{0} loci in the region 5′ to the point of divergence

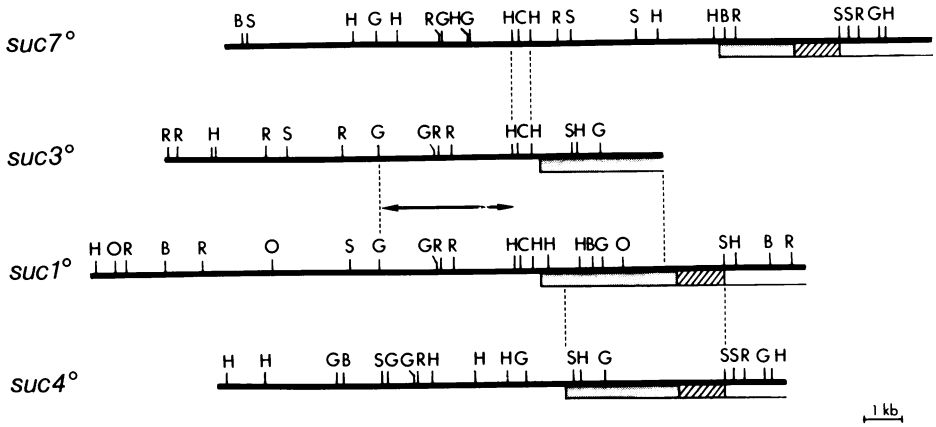

FIG. 5. Comparison of *suc*^{0} loci. Symbols and abbreviations are as in Fig. 1 and 4. Dashed lines indicate relevant regions of homology; X and Y′ sequences at *suc7*^{0} are also homologous to corresponding sequences at other loci. An arrow between the *suc1*^{0} and *suc3*^{0} maps indicates the 3.5-kb region that is identical to cloned *MAL6* DNA (20) with respect to sites for *Bgl*II, *Eco*RI, and *Hin*dIII; *suc1*^{0} and *MAL6* also have identical sites for *Ava*I, *Hpa*I, *Pst*I, and *Pvu*II in this region (data not shown). Not all *Sac*I or *Pst*I sites are shown.

between *suc7*^{0} and *SUC7* is limited to an approximately 0.5-kb element common to *suc1*^{0}, *suc3*^{0}, *suc7*^{0}, *SUC1*, and *SUC7* (and probably also *SUC3*); a series of sites for *Hin*dIII, *Sac*I, *Nco*I, and *Eco*RV is conserved at all these loci (data not shown). The *suc7*^{0} locus also contains X and Y′ elements, but these are separated from the 0.5-kb element by a region of repeated sequences that are not homologous to the other loci. Thus, the termini of chromosomes that can undergo rearrangements resulting in the addition of a *SUC* locus all carry homologous sequences that could have mediated the movement of a *SUC* gene and its flanking sequences from one telomere to another.

DISCUSSION

We characterized the structures of the six *SUC* loci identified in different strains of *Saccharomyces*. Our analysis shows that all of the loci except *SUC2* share extensive regions of homologous sequence flanking the *SUC* structural genes and are located very near telomeres. The homology 5′ to the genes includes X sequences, previously identified as telomere-adjacent sequences (10), with the exception that a substitution at the *SUC1* locus removed most or all of the X sequence. On the 3′ side of the gene, the homology extends through a 4-kb region of flanking sequence and continues into telomere-adjacent Y′ sequences. Thus, the *SUC1*, *SUC3*, *SUC4*, *SUC5*, and *SUC7* genes appear to be located on elements that are embedded in telomere-adjacent sequences. The relative positions of the X and Y′ elements indicate that in each case the telomere lies 3′ to the *SUC* gene. The physical distance between the *SUC* genes and the ends of the chromosomes has not been determined. We thus cannot exclude the unlikely possibility that a gross sequence rearrangement has moved a *SUC* gene and a large region of flanking sequence to a position closer to the centromere.

The boundary of the conserved region 5′ to the *SUC* gene varies depending on the pair of *SUC* loci being compared. The *SUC4* locus has the smallest region of conserved sequence. The *SUC1* and *SUC3* loci share the largest region of homology, which extends into the neighboring *MAL* loci. In addition, the relationship of *SUC1* to the others is unique because the *SUC1* locus appears to carry a substitution of part of the 5′ flanking sequences that are conserved at other loci. On the 3′ side of the *SUC* genes the homology extends into Y′ elements. The Y′ elements distal to the *SUC3*, *SUC4*, *SUC5*, and *SUC7* genes appear to be similar; in contrast, the *SUC1* locus carries a novel variant Y′ element, and the first 131 element distal to the gene appears to be located about 1 kb farther from the *SUC1* gene than is the case at the other loci.

Analysis of the cloned telomeric *suc*^{0} loci showed that the *suc*^{0} loci lack the *SUC* gene and portions of the conserved flanking sequence. The *suc1*^{0}, *suc3*^{0}, and *suc4*^{0} loci are closely related and carry similar X sequences; *suc7*^{0} bears little resemblance to the others. All four of the *suc*^{0} loci, however, display some homology to other *suc*^{0} loci in the regions 5′ to the point of divergence between the *suc*^{0} locus and the corresponding *SUC* locus; such homologies could provide sites for genetic recombination between different chromosome termini that would result in the movement of *SUC* genes and flanking sequences from one chromosome to another. It is important to note that the *suc*^{0} loci examined here were cloned from one particular strain, and the structures of the *suc*^{0} loci that historically were involved in such events may have been somewhat different.

These findings indicate that the dispersal of *SUC* genes to different chromosomes occurred in part by rearrangements of chromosome termini. The data suggest a model for the evolution of the telomeric members of the *SUC* gene family. Comparison of the *SUC* loci, except for *SUC1*, with the *suc1*^{0}, *suc3*^{0}, and *suc4*^{0} loci, suggests that the first telomeric *SUC* locus evolved by the insertion of an approximately 7-kb element containing a *SUC* gene into the telomere-adjacent X sequence of a *suc*^{0} locus similar to *suc1*^{0}, *suc3*^{0}, and *suc4*^{0}. Alternatively, a substitution of such a 7-kb element for a small portion of X sequence may have occurred. The position of the putative insertion or substitution appears to be close to the border of X and Y′; the exact position is not clear because the boundary of the homology between *SUC4* and *suc4*^{0} has not been determined precisely. The resulting *SUC* locus could then move to different chromosomes by recombination between homologous sequences centromere proximal to the point of insertion. Such homologous sequences are present at appropriate positions at all *suc*^{0} loci examined, and simple reciprocal recombination events would generate *SUC* loci with the observed structures, except for *SUC1*, where an additional substitution event must be postulated. Conversion events mediated by the centromere-proximal homology and the Y′ sequences, or

conversion events initiating in the centromere-proximal homologous sequences and extending to the end of the chromosome, similar to those proposed by Dunn et al. (13), would also result in transfer of *SUC* elements to other telomeres. Other models, however, are not excluded by these data; for example, dispersal of such a 7-kb *SUC* gene-containing element to different telomeres could have occurred by repeated transposition of the element into equivalent positions at different suc^0 loci. The observed differences among Y′ sequences at different *SUC* and suc^0 loci do not help to distinguish among models because such differences could have arisen subsequent to events resulting in dispersal of *SUC* genes. An acceptable model must, however, be consistent with a reasonably rapid dispersal of *SUC* genes to different chromosomes. The presence of *SUC* genes at different loci in closely related strains suggests that movement has occurred in recent evolutionary history.

The *SUC2* gene is an unusual member of the *SUC* gene family. It is not embedded in the same flanking sequences as are the other *SUC* genes, nor is it located very close to a telomere, although its genetic map position is near the end of a chromosome (19). Moreover, previous studies have shown that the $suc2^0$ locus is exceptional: its gross structure is identical to that of the *SUC2* locus, and the $suc2^0$ allele is a mutated gene, or pseudogene (3, 7). The evolutionary relationship between *SUC2* and the other *SUC* genes appears to be one of a gene duplication; direct sequence comparison has shown that the homology between *SUC2* and other *SUC* loci includes the structural gene and a 5′ regulatory region distant from the gene (L. Sarokin and M. Carlson, Nucleic Acids Res., in press). We speculate that *SUC2* is the ancestral gene because all the strains that we examined appear to carry *SUC* gene sequences at the *SUC2* locus (*SUC2* or $suc2^0$), while other *SUC* genes are found more rarely (4). The evidence that a gene duplication event occurred adds another variable to the model we propose for the evolution of the telomeric *SUC* loci: the proposed 7-kb element may have evolved at its original telomeric location by the insertion of a *SUC* gene, rather than moving intact to the telomere from another location. It is noteworthy that this 7-kb element contains sequences besides the *SUC* gene that are present only at telomeric *SUC* loci in all the strains examined; in particular, these sequences are not found in the *SUC2* strain S288C.

We also identified a novel variant of the Y′ sequence. Previously, two types of Y′ element have been identified (10). The cloned *SUC1* DNA appears to carry a third type not previously described. A characterization of this distinct member of the Y′ family will be reported elsewhere (F. J. Eng and M. Carlson, manuscript in preparation).

Genetic analysis showed that *SUC1* and *MAL1* are tightly linked and located near the telomere of chromosome VII, but their order relative to one another on the genetic map was not determined (9, 19). The identification of sequences homologous to cloned *MAL* DNA located toward the centromere from the *SUC1* gene suggests that the correct order is centromere-*MAL1*-*SUC1*. Analogous findings suggest that the order of the tightly linked *SUC3* and *MAL3* loci on chromosome II (19) is centromere-*MAL3*-*SUC3*.

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