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Molecular Evolution of the Telomere-Associated *MAL* Loci of Saccharomyces

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Manuscript received December 5, 1988
Accepted for publication February 11, 1989

ABSTRACT

The *MAL* gene family of Saccharomyces consists of five multigene complexes (*MAL1*, *MAL2*, *MAL3*, *MAL4* and *MAL6*) each of which encodes maltose permease (GENE 1), maltase (GENE 2) and the *trans*-acting *MAL*-activator (GENE 3). Four of these loci have been mapped and each is located at or near the telomere of a different chromosome. We compare the physical structure of the *MAL* loci and their flanking sequences. The *MAL* loci were shown to be both structurally and functionally homologous throughout an approximately 9.0-kb region. The orientation of the *MAL* loci was determined to be: CENTROMERE ... GENE 3–GENE 1–GENE 2 ... TELOMERE. Telomere-adjacent sequences were found flanking GENE 2 of the *MAL1*, *MAL3* and *MAL6* loci. No common repeated elements were found on the centromere-proximal side of all of the *MAL* loci. These results suggest that, during the evolution of this polygenic family, the *MAL* loci translocated to different chromosomes via a mechanism that involved the rearrangement(s) of chromosome termini.

FERMENTATION of the disaccharide maltose by the Saccharomyces yeasts requires the presence of any one of a family of five unlinked loci (*MAL1*, *MAL2*, *MAL3*, *MAL4* and *MAL6*) [reviewed by BARNETT (1976, 1981)]. Four of the five *MAL* loci have been genetically mapped and are located at or near a telomere: *MAL1*, right arm chromosome *VII* (CELENZA and CARLSON 1985); *MAL2*, right arm chromosome *III*; *MAL3*, right arm chromosome *II*; and *MAL4*, right arm chromosome *XI* (MORTIMER and SCHILD 1980). *MAL6* is linked to chromosome *VIII*, however its exact map position is unknown (DUBIN 1987). A similar genomic arrangement is observed in the *SUC* gene family encoding invertase (CELENZA and CARLSON 1985; MORTIMER and SCHILD 1980). In fact, the *MAL1* and *MAL3* loci are tightly linked to the *SUC1* and *SUC3* loci, respectively.

Genetic and physical analyses of strains containing each of the *MAL* loci show that the *MAL* loci are highly sequence homologous (MICHELS and NEEDLEMAN 1983; NEEDLEMAN and MICHELS 1983; MICHELS and NEEDLEMAN 1984). Physical comparison of the cloned *MAL1* and *MAL6* loci by restriction mapping and Southern analysis reveals extensive homology over an approximately 9.0-kb region (CHARRON, DUBIN and MICHELS 1986). Functional analysis of this region from both loci demonstrates the presence of three genes (COHEN *et al.* 1984; NEEDLEMAN *et al.* 1984; COHEN *et al.* 1985; DUBIN *et al.* 1985; CHARRON, DUBIN and MICHELS 1986; CHANG *et al.* 1988; Y. S. CHANG, R. A. DUBIN, E. PERKINS, C. A. MICHELS and R. B. NEEDLEMAN, unpublished data). GENE 1 appears to encode maltose permease; GENE 2 encodes maltase and GENE 3 encodes a positive *trans*-acting regulator of the structural genes and is referred to as the *MAL* activator.

In this study we extend our comparative analysis of the *MAL* loci to *MAL2*, *MAL3* and *MAL4*, as well as to the DNA sequences flanking each of the five *MAL* loci. Our results demonstrate that all of the *MAL* loci are both structurally and functionally homologous throughout an approximately 9.0-kb region containing the three genes encoding the fermentative enzymes and the activator protein. Additional sequence homology extending beyond this 9.0-kb region to the centromere-proximal side of the *MAL2* and *MAL4* loci and the *MAL3* and *MAL6* loci is detected. The orientation of the *MAL* loci was determined to be: CENTROMERE ... GENE 3–GENE 1–GENE 2 ... TELOMERE. Telomere-adjacent sequences are found in the region flanking GENE 2 of the *MAL1*, *MAL3* and *MAL6* loci. No common repeated elements flank the centromere-proximal region of all the *MAL* loci. The implication of these results with regard to the mechanism of translocation of the *MAL* loci is discussed.

MATERIALS AND METHODS

Strains, growth conditions and DNA analysis: Table 1 lists the strains utilized in this study. Growth of yeast strains and determination of maltose fermentation phenotype were as previously described (CHARRON, DUBIN and MICHELS 1986).

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TABLE 1

List of yeast strains

Strain	Genotype	Source
600-1B	*MAT***a** *MAL1 SUC1 ura3-52 leu2-3,112*	CHARRON and MICHELS (1988)
MCY101-3A	*MAT***a** *MAL2 mal11,MAL12,mal13 ura3-52 ade1 leu2-3,112*	This report
1412-4D	*MAT***a** *MAL3 MAL11-2,MAL12,mal13Δ SUC3 MGL2 MGL3 ade2*	MICHELS and NEEDLEMAN (1983)
48-2C	*MATα MAL3 mal11,MAL12,mal13 ade1 lys2*	R. NEEDLEMAN
MCY102-5A	*MATα MAL3 mal11,MAL12,mal13 ura3-52*	This report
MCY100-3A	*MATα MAL4-C mal11,MAL12,mal13 ura3-52 lys*	CHARRON and MICHELS (1987)
MCY100-2C	*MATα MAL4-C mal11,MAL12,mal13 ura3-52 leu2-3,112 ade*	CHARRON and MICHELS (1987)
236-2A	*MAT***a** *mal11,mal12Δ,MAL13 leu2-3,112 lys2*	CHARRON and MICHELS (1988)
345-4A	*MAT***a** *mal11,mal12Δ,MAL13 leu2-3,112 trp1 ade ura3-52*	CHARRON and MICHELS (1988)
53-2C[a]	*MATα mal11,mal12Δ,MAL13 met*	CHARRON, DUBIN and MICHELS (1986)
328-4A	*MATα mal11,MAL12,mal13 ura3-52 trp1 ade met14*	CHARRON and MICHELS (1988)
303-3A	*MATα mal11,MAL12,mal13 leu2-3,112 ade1*	CHARRON and MICHELS (1988)
340-2B	*MAT***a** *MAL11-2,MAL12,*mal13Δ *ura3-52 trp1 lys met*	CHARRON and MICHELS (1988)
JC27	*MATα MAL11-2,MAL12,mal13Δ MAL31,MAL32,mal33Δ leu2-3,112 his*	FEDEROFF et al. (1982)

The *MAL* nomenclature used here is based on the previous studies of *MAL* loci. A dominant *MAL* locus having all three functional genes (GENE 1, GENE 2 and GENE 3) is given a single digit number such as, *MAL2* or *MAL6*. The genotype of the partially functional *MAL1* and *MAL3* alleles is completely specified so to indicate the functional state of each of the three genes. The genotype is based on results reported in CHARRON and MICHELS (1988) and CHARRON (1988).

[a] Strain 53-2C contains a functional gene encoding only maltase which is unlinked to *MAL1* but which we have as yet not mapped to a specific linkage group.

Plasmid, yeast and phage DNAs were prepared according to NEEDLEMAN *et al.* (1984) and CHARRON, DUBIN and MICHELS (1986). Specific details of the procedure used for Southern gel analysis may be found in MICHELS and NEEDLEMAN (1984).

Yeast transformation and plasmid rescue: Yeast transformations were carried out by the method of ITO *et al.* (1983) using lithium acetate. Transformants were screened for functional ARS sequences by assaying plasmid stability.

All plasmids generated were assayed for functional *MAL* genes by the ability to complement standard tester strains carrying one of the following partially functional alleles of *MAL1*: *MAL13 mal11 mal12Δ* (*MAL1p* allele); *mal13Δ MAL11-2 MAL12* (*MAL1g* allele) and/or *mal13 mal11 MAL12* (*mal1*^{0} allele) (MICHELS and NEEDLEMAN 1983; CHARRON, DUBIN and MICHELS 1986; CHARRON and MICHELS 1988; see Table 1). Approximately 50 transformants were assayed for their maltose fermentation phenotype.

The method of plasmid rescue was used to isolate several of the *MAL* loci and their flanking DNA in order that the genomic origin of the isolated sequences could be unambiguously known. Plasmid rescue involves the following steps: (1) targeted integration of a shuttle vector to a site linked to the desired sequence, (2) genetic and physical demonstration of the actual site of integration, and (3) isolation of the integrated vector along with flanking DNA sequences. Plasmids pMJC6ΔC, pY6-RΔC, pY6ΔCΔH and pGΔC were used for site-directed integration (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1983) into several of the *MAL* loci following linearization with *Cla*I, *Bgl*II, *Hpa*I and *Cla*I, respectively, prior to transformation (Figure 1, see asterisk (*) for site of cleavage; DUBIN *et al.* 1986; CHARRON and MICHELS 1988; DUBIN *et al.* 1988). Linkage of the plasmid marker (*URA3*) to a particular *MAL* locus was determined using both physical and genetic analysis similar to that described in CHARRON, DUBIN and MICHELS (1986). *MAL*-linked DNA sequences were isolated from pY6-RΔC transformed strains following digestion with *Bam*HI; from pY6ΔCΔH transformed strains following digestion with *Hin*dIII, *Bam*HI or *Sal*I; from pMJC6ΔC transformed strains following digestion with *Bam*HI; and from pGΔC transformed strains following digestion with *Kpn*I or *Sal*I.

Cloning the *MAL3* locus: *MAL3* sequences were isolated from strains 48-2C and MCY102-5A. Both strains contain the identical *MAL3* locus since both are derived from the *MAL3* strain 1412-4D of the Berkeley Yeast Stock Center. Genomic DNA from strain 48-2C, partially restricted with *Sau*3A, was ligated into EMBL3 *Bam*HI arms, packaged and amplified as described in CHARRON, DUBIN and MICHELS (1986). The resultant library was screened with the *MAL6*-derived probe pD-1 and one phage clone, λMJC3.1, was isolated (Figure 2). Combined physical and genetic analysis of strain 1412-4D carrying the *MAL3* locus has shown that three *Hin*dIII fragments homologous to plasmid probe pD-1 are linked to the *MAL3* locus (MICHELS and NEEDLEMAN 1983). One of these (approximately 7.3 kb) is highly homologous to plasmid pD-1. The other two fragments hybridize weakly to the probe and are smaller, approximately 5 kb. Analysis of the yeast insert of phage clone λMJC3.1 shows that it contains a 7.5-kb *Hin*dIII fragment that is highly homologous to pD-1, thus suggesting linkage to the *MAL3* locus. Plasmid subcloning and complementation studies carried out on phage. λMJC3.1 insert DNA demonstrate the presence of functional *MAL31*, *MAL32*, and *MAL33* genes (described in RESULTS and Table 2). Based on these results, phage λMJC3.1 contains the *MAL3* locus (Figure 2). The DNA sequences flanking *MAL3* were isolated by plasmid rescue and the restriction endonuclease map shown in Figure 2 is a composite of the restriction maps of all the rescued plasmids as well as that of phage λMJC3.1. The isolation of the flanking sequences to the right of *MAL32* was accomplished by the rescue of plasmid pSRH3B from strain MCY102-5A transformed with pY6ΔCΔH (Figure 1) as described above. The yeast insert contained in plasmid pSRHL3B is shown in Figure 2.

Flanking DNA sequences to the left of *MAL33* were also isolated by plasmid rescue but utilizing plasmid pGΔC (Figure 1) as described above. A detailed account of this work is being prepared for publication elsewhere. Plasmid pGΔC surprisingly was found to integrate at two distinct but tightly linked sites in strain MCY102-5A. Both sites of integration

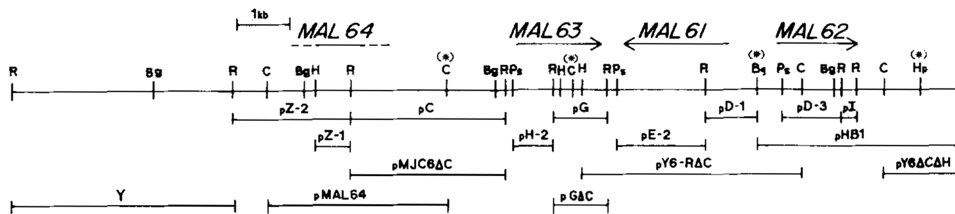

FIGURE 1.—Restriction endonuclease map of the *MAL6* locus from strain CB11 and subclones used in this study. A partial restriction endonuclease map of the *MAL6* locus along with the locations of the *MAL61*, *MAL62*, *MAL63* and *MAL64* genes is presented. Direction of transcription of *MAL61*, *MAL62* and *MAL63* is shown. All subclones shown are contained in plasmid pBR325 except pY6ΔCΔH (in YIp5Δ*Hin*dIII), pY6-RΔC (in YIp5), pMJC6ΔC (in YIp5Δ*Cla*I), and pGΔC (in YIp5Δ*Cla*I) (CHARRON, DUBIN and MICHELS 1986; CHARRON and MICHELS 1988; DUBIN *et al.* 1988). Probe *Y* was purified by extraction from an agarose gel and was obtained from *Eco*RI digested plasmid pBam11 (DUBIN *et al.* 1988). The symbol (*) represents the site of linearization of plasmids pY6ΔCΔH, pY6-RΔC, pMJC6ΔC, and pGΔC used in site-directed integration experiments. Restriction endonucleases are abbreviated as follows: Bg, *Bgl*II; C, *Cla*I; H, *Hin*dIII; Hp, *Hpa*I; Ps, *Pst*I; R, *Eco*RI.

TABLE 2

Functional homology of the *MAL* loci

Plasmid	*MAL* GENE(s) contained	Fragment kb (end points)	Ability to complement maltose nonfermenting strains of the given genotype: *MAL13 mal11 mal12*Δ (MAL1p allele)	*mal13*Δ *MAL11-2 MAL12* (MAL1g allele)	*mal13 mal11 MAL12* (*mal1*^{0} allele)
pM1.2F	*MAL13*	7.1 (*Bgl*II-*Sal*I)	−	+	−
pMJC1ΔH	*MAL11 MAL12*	7.3 (*Hin*dIII-*Hin*dIII)	+	−	−
pMJC2B	*MAL23 MAL21*	7.7 (*Bam*HI-*Cla*I)	−	+	+
pMJC2ΔH	*MAL21 MAL22*	7.6 (*Hin*dIII-*Hin*dIII)	+	−	−
pM3.1C	*MAL33*	3.1 (*Sal*I-*Sal*I)	−	+	−
pM3.1A	*MAL31 MAL32*	7.3 (*Hin*dIII-*Hin*dIII)	+	−	−
pMJC4B	*MAL43 MAL41*	9.0 (*Bam*HI-*Cla*I)	−	+	+
pM43BS	*MAL43*	5.8 (*Bam*HI-*Sal*I)	−	+	−
pMJC4ΔH	*MAL41 MAL42*	7.6 (*Hin*dIII-*Hin*dIII)	+	−	−
pDF-1	*MAL63*	2.6 (*Bgl*II-*Sal*I)	−	+	−
pY6	*MAL61 MAL62*	7.3 (*Hin*dIII-*Hin*dIII)	+	−	−
p21-40	*MAL63 MAL61*	6.8 (*Bgl*II-*Bgl*II)	−	+	−

As indicated in columns two and three, clones containing each of the genes from the *MAL* loci were tested for the ability to complement maltose nonfermenting tester strains. Plasmid pM1.2F (CHARRON, DUBIN and MICHELS 1986) contains the indicated insert in YEp24. The *MAL3* sequences in plasmid pM3.1C (Figure 2) and *MAL6* sequences in plasmid p21-40 (NEEDLEMAN *et al.* 1984) are in YEp13. *MAL3* and *MAL6* sequences in plasmids pM3.1A (Figure 2) and pY6 (NEEDLEMAN *et al.* 1984), respectively, are in YIp5. The *MAL43* plasmid pM43BS (CHARRON and MICHELS 1987) and the *MAL63* plasmid pDF-1 contain the indicated yeast sequence in pLC544. Plasmids pMJC2B and pMJC4B were isolated from strains MCY101-3A and MCY100-3A, respectively, using plasmid rescue techniques described previously (CHARRON and MICHELS 1988). Both plasmids contain an 800-bp *Bgl*II-*Cla*I fragment derived from *MAL62*. Plasmids pMJC1ΔH, pMJC2ΔH and pMJC4ΔH were isolated from strains 600-1B, MCY101-3A and MCY100-2C, respectively, using plasmid rescue techniques. All three plasmids contain the 2.4-kb *MAL6*-derived *Cla*I-*Hin*dIII fragment of plasmid pY6ΔCΔH. The genotypes of the strains used for the complementation tests are described in detail in CHARRON and MICHELS (1988). The particular strains used were: 345-4A and 236-2A (*mal11 mal12*Δ *MAL13*); 328-4A and 303-3A (*mal11 MAL12 mal13*Δ); and 340-2B (*MAL11-2 MAL12 mal13*Δ) and JC27 (*MAL11-2 MAL12 mal13*Δ; *MAL31 MAL32 mal33*Δ).

are indicated by arrows below the composite *MAL3* restriction map in Figure 2 (see site 1 and site 2). One integrative transformant of each class was retained for further analysis. Plasmids pER3-1K, pER3-2K and pER3-2S were isolated from these transformants by digestion of total genomic DNA with *Kpn*I, *Kpn*I and *Sal*I, respectively, followed by plasmid rescue according to procedures described above. The yeast insert contained in each of these plasmids is indicated in Figure 2.

Construction of plasmid pL5-15: In order to determine the orientation of the *MAL2* locus with respect to the centromere and telomere, plasmid pL5-15 was constructed by

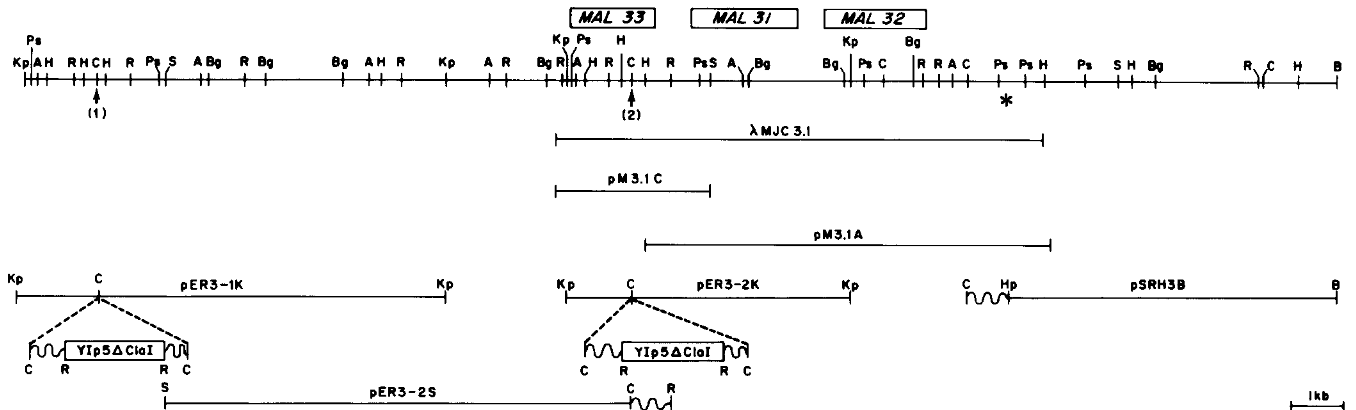

FIGURE 2.—Restriction endonuclease map of the *MAL3* locus and flanking DNA sequences. The map shown is a composite drawn from restriction endonuclease mapping of the yeast inserts in phage λMJC3.1 and plasmids pSRH3B, pER3-1K, pER3-2K and pER3-2S. The *MAL3* locus of strain 48-2C was cloned into phage EMBL3 and phage clone λMJC3.1 is shown. Plasmid pM3.1C contains the 3.1 kb *Sal*I fragment from λMJC3.1 in YEp13 and plasmid pM3.1A contains the 7.5 kb *Hin*dIII fragment of λMJC3.1 in YIp5. Plasmids pSRH3B, pER3-1K, pER3-2K and pER3-2S were isolated from strain MCY102-5A by plasmid rescue (MATERIALS AND METHODS; C. A. MICHELS and E. READ, unpublished results). The asterisk (*) and the arrows (↑) below the map indicate the sites of integration of the plasmids used in the plasmid rescue. The symbol (∿) indicates *MAL6*-derived sequences. Because of the method of isolation, plasmids pER3-1K and pER3-2K both contain a complete copy of plasmid pGΔC in addition to the *MAL3*-derived sequences shown above. The complex organization of the *MAL3* sequences in these plasmids is indicated. Restriction enzymes are abbreviated as in Figure 1 with the following additions: A, *Ava*I; B, *Bam*HI; Kp, *Kpn*I; S, *Sal*I.

ligating the *Hin*dIII/*Sal*I digested *HMR* plasmid p15C (KLAR *et al.* 1981) to the *MAL63* gene disruption plasmid pDM3 (CHARRON, DUBIN and MICHELS 1986; CHANG *et al.* 1988) which was partially restricted with *Hin*dIII and completely digested with *Sal*I. Plasmid pL5-15 contains the 4.1-kb *Hin*dIII/*Eco*RI fragment from plasmid p15C containing the *HMR* locus, the 900-bp *Hin*dIII/*Sal*I fragment from the 3′ end of the *MAL63* gene derived from plasmid pDM3 along with some vector sequences and the 1.1-kb *Hin*dIII fragment from YIp30 containing the *URA3* gene. The organization of these fragments is shown in Figure 4. Plasmid pL5-15 was digested with *Eco*RI prior to transformation of strain MCY101-3A to Ura$^+$.

RESULTS

Structural and functional comparison of the coding region of the *MAL* loci: FEDEROFF *et al.* (1982) describe the isolation of a 12.5-kb DNA fragment which was shown to contain the *MAL6* locus (NEEDLEMAN and MICHELS 1983). In a previous report, we extended the size of this cloned region an additional 9 kb by isolating chromosomal fragments flanking the *MAL6* locus using the technique of plasmid rescue which involves the recovery from the genome of chromosomally integrated plasmids (described in MATERIALS AND METHODS; DUBIN *et al.* 1988). This same technique was used to isolate the *MAL2* and *MAL4* loci along with flanking DNA sequences from the strains listed in Table 1 (see MATERIALS AND METHODS; CHARRON and MICHELS 1987). The isolation of the *MAL1* locus has been described previously (CHARRON, DUBIN and MICHELS 1986). The *MAL3* locus and flanking DNA sequences were isolated both from a genomic phage library and by genomic plasmid rescue as described in the MATERIALS AND METHODS (see Figure 2). Figure 3 shows the restriction map of each of these loci along with their flanking DNA sequences. Indicated above the map is the position of the genes encoding maltose permease (GENE 1), maltase (GENE 2) and the *MAL* activator (GENE 3) that have been identified at the *MAL1* and *MAL6* loci.

Based on a comparison of the restriction maps, the cloned sequences appear to be highly conserved over approximately 9.0 kb of DNA including the coding regions plus about 2 kb of noncoding sequences beyond GENE 2. Only a few restriction site polymorphisms and the presence of a small insert into *MAL3* in the region between GENE 1 and GENE 3 distinguish the different loci (Figure 3). The results of Southern analysis using probes spanning the entire *MAL6* locus (see Figure 1) confirm that all of the *MAL* loci are highly sequence homologous over this 9.0 kb region. In addition, homology between the *MAL3* and *MAL6* loci extends approximately 4.5 kb beyond GENE 3 into flanking sequences and homology between the *MAL2* and *MAL4* loci extends approximately 3 kb beyond GENE 3 into flanking sequences. These observations will be discussed further below. The vertical dotted lines indicate the approximate boundary of the homology (Figure 3).

In order to identify both the locus position and the gene function of each *MAL* gene we have proposed a system of nomenclature that utilizes a two digit gene number (NEEDLEMAN *et al.* 1984; DUBIN *et al.* 1985; CHARRON, DUBIN and MICHELS 1986; DUBIN *et al.* 1988). The first digit designates the locus position and the second digit indicates the gene function as presented in Figure 3 and described in the Introduc-

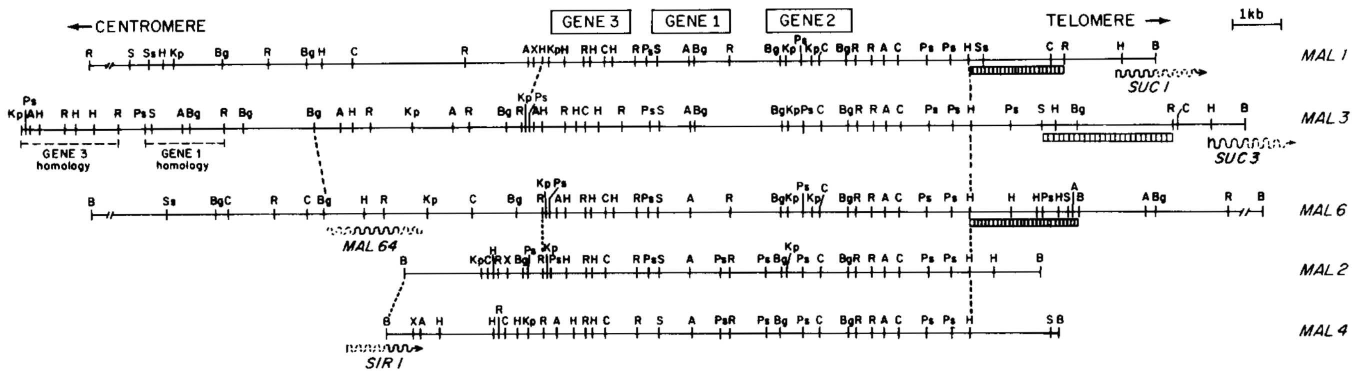

FIGURE 3.—Comparison of the *MAL* loci. The restriction endonuclease map of the *MAL1*, *MAL2*, *MAL3*, *MAL4* and *MAL6* loci and their flanking sequences. The approximate location of the three genes needed for maltose fermentation are diagrammed above the maps. The boundaries of homology between the *MAL* loci are indicated by vertical dotted lines. Homology to telomere-derived *X* sequences was detected using plasmid YRp120 (CHAN and TYE 1983) and regions of homology are diagrammed with vertical hatched bars. Regions of homology to *SUC* sequences (plasmid pRB117 containing the 5′ region of the *SUC2* gene; CARLSON and BOTSTEIN 1982); to *SIR* sequences (plasmid pJH570 containing the entire *SIR1* gene; IVY, KLAR and HICKS 1986) and to *MAL64* (DUBIN *et al.* 1988) are indicated by wavy lines below the restriction map with the arrowhead indicating the direction of transcription where known. Regions showing sequence homology to GENE 1 and GENE 3 probes, located on the centromere-proximal side of *MAL3*, are indicated below the restriction map as a dashed line. Recognition sites for restriction endonucleases are abbreviated as in Figures 1 and 2 with the following additions: X, *Xho*I. Not all *Cla*I, *Hin*dIII, *Kpn*I, or *Pst*I sites are shown in *MAL3* flanking DNA sequences.

tion. Thus, for example, *MAL21* is the maltose permease gene present at the *MAL2* locus, and *MAL32* is the maltase structural gene contained within the *MAL3* locus.

Plasmid complementation studies were performed using cloned sequences from each of the *MAL* loci to determine if the *MAL* loci have remained functionally homologous. The results of this analysis are presented in Table 2. Each plasmid was capable of functioning as an episomal plasmid either because a yeast 2-micron vector or a yeast ARS vector was used, or as was found in this study and in previous work, the cloned *MAL* sequences themselves provided the ARS element. Detailed analysis of the *MAL6* locus localized an ARS element to the 500-bp *Hin*dIII-*Eco*RI fragment between the 3′ end of the *MAL61* and the *MAL63* genes (Y. SYLVESTRE and C. A. MICHELS, unpublished data). The plasmids were transformed into strains carrying partially functional alleles of *MAL1*. A detailed structural and functional analysis of these alleles is presented in CHARRON and MICHELS (1988) and Table 2 indicates the genotype of each strain as deduced from this analysis.

In summary, the results presented in Table 2 demonstrate that the *MAL* loci are not only highly homologous on the sequence level but have maintained functional homology. The structural genes (GENE 1 and GENE 2) from each *MAL* locus complement the *MAL13* gene of the *MAL1p* locus. With one exception (plasmid p21-40), the activator gene product of each of the loci is capable of activating the expression of the *MAL11-2* and *MAL12* genes of the *MAL1g* allele and the *MAL12* gene of the *mal1*^{0} allele. The negative results with plasmid p21-40 have not been investigated further.

Orientation of the *MAL* loci: *Homology to telomere adjacent sequences and to SUC sequences in MAL flanking DNA*: All of the *MAL* loci, except *MAL6*, have been mapped in the Saccharomyces genome and have been shown to be near a chromosomal telomere (MORTIMER and SCHILD 1980; CELENZA and CARLSON 1985). Studies done on the *MAL1* locus and its alleles have shown that telomere adjacent *X* sequences, *SUC1* and *suc1*^{0} and, in the case of the *MAL1g* allele, *Y*′ sequences flank one side of the locus (adjacent to GENE 2) and not the other side of the locus (CARLSON, CELENZA and ENG 1985; CHARRON and MICHELS 1988). Additionally, these reports demonstrate tight linkage between the *MAL3* and *SUC3* loci. With these results in mind we next determined if any of the other sequences cloned from the *MAL* loci contain homology to known telomere adjacent sequences (*i.e.*, *X*, *Y*′ and *SUC*). The results of Southern analyses are summarized in Figure 3. Telomere adjacent *X* sequences are found flanking GENE 2 of the *MAL1*, *MAL3* and *MAL6* loci and the approximate location is indicated. Significant homology to *Y*′ sequence was not detected in the cloned sequences from these three loci but this result is not surprising. *Y*′ sequences are found immediately next to the chromosomal terminus. Both the *MAL1* and *MAL3* loci used in our studies have linked *SUC* genes (*SUC1* and *SUC3*, respectively) and these map closer to the telomere than does the *MAL* locus (CARLSON, CELENZA and ENG 1985). In fact, sequences derived from the *SUC1* and *SUC3* loci are present in the GENE 2 flanking DNA of *MAL1* and *MAL3*, respectively. The *MAL1-SUC1* physical linkage was previously reported (CHARRON, DUBIN and MICHELS 1986). The absence of significant *Y*′ homology in the cloned *MAL6* sequences might be explained

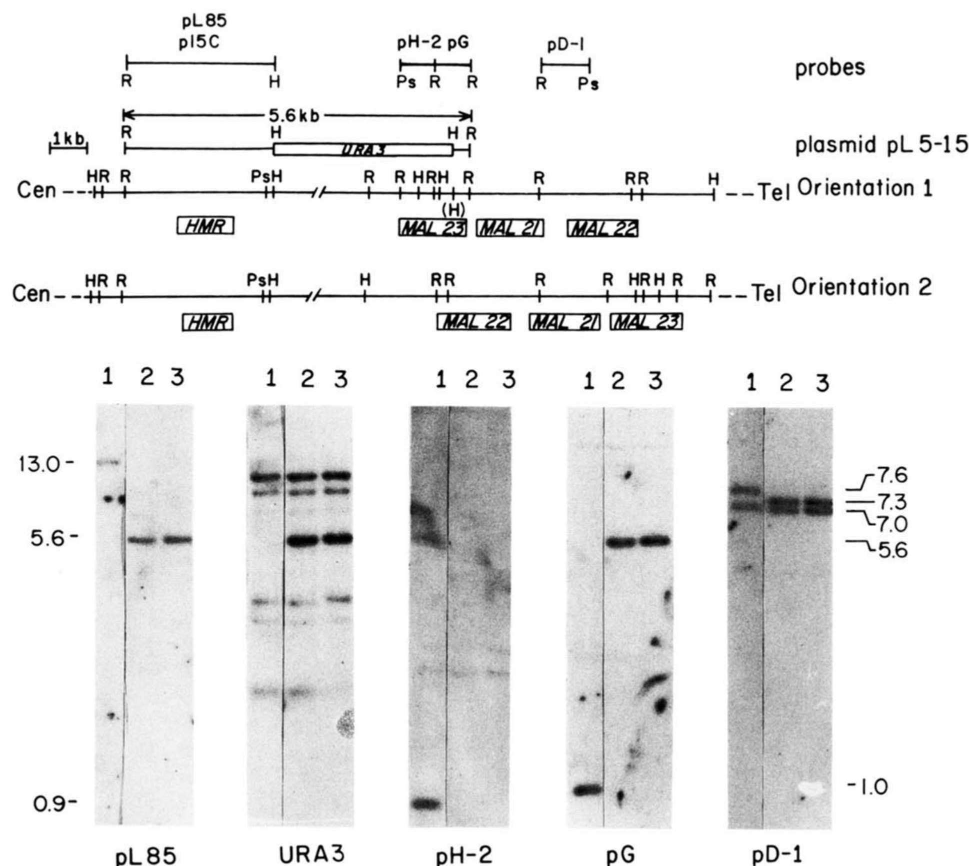

FIGURE 4.—Orientation of *MAL2* by chromosomal deletion. Location of *HMR*, *MAL21*, *MAL22* and *MAL23* are shown in proposed ORIENTATIONS 1 and 2. Centromere and telomere are abbreviated as Cen and Tel respectively. The yeast insert of disruption plasmid pL5-15 is diagrammed above the map along with the *HMR*-linked plasmids pL85 and p15C and the *MAL6*-linked plasmids pG, pH-2 and pD-1 (which are drawn above the regions of *MAL2* to which they are homologous). Results of Southern gel transfer analysis of strains MCY101-3A (lane 1), L5-15#1 (lane 2) and L5-15#17 (lane 3) are shown. Panels #1 through #4 contain *Eco*RI digested DNA probed with pL85, *URA3*, pH-2 and pG, respectively. Panel #5 contains *Hin*dIII digested DNA probed with pD-1. Sizes of fragments homologous to the probes used are indicated as kilobasepairs.

by the fact that the *MAL6* locus originated in *S. carlsbergenesis* strains and it has been shown the *Y'* homology is not highly conserved in species of *Saccharomyces* other than *S. cerevisiae* (CHAN and TYE 1983).

No significant homology to any of the telomere associated probes was detected in the cloned *MAL2* and *MAL4* flanking DNA sequences. Both loci are located on smaller yeast chromosomes and, according to ZAKIAN and BLANTON (1988), *X* and *Y'* sequences appear to be absent from several of the smaller *S. cerevisiae* chromosomes (chromosomes *I*, *III*, *VI* and *XI*) but this is strain dependent.

In summary, three of the *MAL* loci contain known telomere adjacent DNA sequences flanking one side of the locus (adjacent to GENE 2) and not the other. These results allow us to conclude that: (1) *MAL6*, like the other four *MAL* loci, maps close to a chromosome terminus; and (2) the orientation of *MAL1*, *MAL3* and *MAL6* is CENTROMERE . . . GENE 3–GENE 1–GENE 2 . . . TELOMERE.

Orientation of MAL2 by chromosomal deletion: In order to determine the orientation of the *MAL2* locus with respect to the centromere and telomere, disruption plasmid pL5-15 was constructed to delete the region of chromosome *III* from *HMR* to the *MAL23* gene (see MATERIALS AND METHODS; Figure 4). By determining which *MAL2* sequences are deleted during the transplacement we will be able to determine the orientation of *MAL2*. If ORIENTATION 1 is correct, disruption by the 5.6-kb *Eco*RI fragment of plasmid pL5-15 will leave the *MAL21* and *MAL22* genes intact but will delete a portion of the *MAL23* gene resulting in a nonfermenting strain which should be complemented by a *MAL13 mal11 mal12* strain. As diagrammed in Figure 4, the 5.6-kb *Eco*RI disruption fragment of plasmid pL5-15 will not mediate any viable transplacement events if ORIENTATION 2 is correct. Transplacement events will be abortive since such events will produce abnormal chromosomal termini (R. ROTHSTEIN, personal communication). Thus, any stable Ura^+ transformants that are formed will have resulted from other types of rearrangement/recombination events not involving the *HMR–MAL2* region such as gene conversions of the *ura3-52* mutation.

Strain MCY101-3A, a haploid strain carrying the *MAL2* locus, was transformed using plasmid pL5-15 and stable Ura^+ transformants were isolated. These were tested for their ability to ferment maltose. Two pL5-15 transformants (L5-15#1 and L5-15#17), out of 228 screened, did not ferment maltose. The 226 maltose fermenting transformants were found to contain plasmid pL5-15 as an episomal plasmid. Results of Southern analysis on the 2 nonfermenting transformants using *MAL63*-derived probes, pH-2 and pG; the *HMR*-specific probe, pL85 (STRATHERN *et al.*

1980); a fragment from the *URA3* gene and pD-1 (Figure 1) demonstrate the expected deletion/disruption for ORIENTATION 1 has occurred, that is, that *MAL23* sequences have been deleted from strain MCY101-3A leaving the *MAL21* and *MAL22* genes intact (Figure 4). Additionally, in these transformants the *HMR* locus is now tightly linked to *MAL* sequences as evidenced by the fact that transformants L5-15#1 and L5-15#17 contain a 5.6 kb *Eco*RI fragment homologous to pL85, pG and *URA3* sequences. Strains L5-15#1 and L5-15#17 lack the 900 bp pH-2 homologous *Eco*RI fragment seen in MCY101-3A derived from the *MAL23* gene. The 13.0-kb pL85 homologous *Eco*RI fragment containing the *HMR* locus and the 1.0-kb pG-homologous *Eco*RI fragment derived from the *MAL23* gene shift in size to that of the pL5-15 disruption fragment, 5.6 kb. When these strains are digested with *Hin*dIII and probed with pD-1 a decrease of approximately 300 bp is detected in the disruption strains (7.6 kb to 7.3 kb). This can be accounted for by the presence of a *Hin*dIII restriction site polymorphism between *MAL6* (donor sequences) and *MAL2* (acceptor sequences) as shown in Figure 4. The GENE 3-homologous *Eco*RI-*Hin*dIII fragment used in the construction of plasmid pL5-15 was derived from *MAL63* and the *MAL23* gene lacks a *Hin*dIII site at this position. Strains L5-15#1 and L5-15#17 were further analyzed and shown to be complemented by strain 53-2C which lacks GENE 1 function (CHARRON, DUBIN and MICHELS 1986; CHARRON and MICHELS 1988).

Only the *MAL4* locus has not been unambiguously oriented with regard to the telomere but, based on our isolation of the *SIR1* gene on the same fragment as *MAL43* and on results obtained in other laboratories (described below) we have diagrammed the *MAL4* locus in Figure 3 in the same orientation as the other *MAL* loci.

In summary, all of the *MAL* loci are found next to a chromosome terminus and all appear to be oriented as follows: CENTROMERE . . . GENE 3-GENE 1-GENE 2-[*SUC*] . . . TELOMERE.

Centromere-proximal sequences flanking the *MAL* loci: One of the proposed mechanisms leading to the formation of polygenic families of loci, such as *MAL* and *SUC*, is an *inter*-chromosomal recombination process involving homologous sequences proposed to be present at the centromere-proximal side of each member of the polygenic family. Telomere-associated *X* sequence is found linked to each *SUC* locus on the centromere proximal side and it has been suggested that recombination between these *X* sequences and *X* sequences located at other telomeres was responsible for the translocation of the *SUC* loci (CARLSON, CELENZA and ENG 1985). For this reason, it became important to analyze the region of each *MAL* locus flanking GENE 3 for sequences common to all of the loci.

One of the four *MAL1* alleles has been found in all strains examined by our laboratory, and for this reason it has been proposed that it is the progenitor *MAL* locus. With this in mind we used probes containing the three *Eco*RI fragments on the centromere proximal side of *MAL13* (Figure 3; CHARRON, DUBIN and MICHELS 1986; CHARRON and MICHELS 1988) to probe all the other cloned *MAL* sequences. No sequence homology was detected, suggesting that these sequences are unique to *MAL1*.

A fourth *MAL* gene has been identified at the *MAL6* locus (*MAL64*). This gene has been shown to be functional in maltose fermentation only in *MAL6* constitutive mutants (DUBIN *et al.* 1988). DUBIN *et al.* (1988) show that *MAL64* constitutive mutations lie in a region which shows significant sequence homology to *MAL63* and *MAL61* and appears to represent a tandem duplication of these sequences. Probes derived from this region (pZ-1, pZ-2, pMAL64 and *Y* in Figure 1) were used to examine the other *MAL* loci for potential *MAL64* homologs and only in *MAL3* was significant hybridization detected. Plasmid pMAL64, the *Cla*I fragment containing the *MAL64* gene (Figure 1; DUBIN *et al.* 1988), hybridizes well to the region flanking *MAL33* indicated in Figure 3. Like *MAL64*, this region exhibits weak but significant homology to the *MAL63* probes pH-2 and pG (Figure 1). In addition, the restriction endonuclease map is very similar to that of *MAL64*. Thus we propose that a *MAL3* equivalent of *MAL64*, *MAL34*, is present at this site. Sequences which weakly hybridize to pZ-2 were found in the region upstream of *MAL23* but these have not been investigated further.

Adjacent to the *MAL3* locus, approximately 5 kb from the *MAL33* gene, is a region whose restriction endonuclease map is largely identical to that of the GENE 3 and GENE 1 regions of the *MAL* loci. *MAL6*-derived probes for the GENE 3 and GENE 1 region hybridize well to this region. GENE 2 sequences appear to be absent. The characteristic restriction endonuclease map of GENE 2 is not seen and plasmid pD-1 hybridizes poorly to the region. In summary, it is clear that homology between the *MAL6* and *MAL3* loci extends beyond GENE 3 to include the linked *MAL64* (*MAL34*) gene and that additional sequences which are highly homologous to the *MAL* genes are located immediately centromere proximal to *MAL3* and in tandem orientation to *MAL3*. Although linked *MAL*-homologous sequences are not seen on the centromere proximal side of *MAL64*, it is clear that *MAL3* and *MAL6* are more evolutionarily related to each other than to *MAL1*, *MAL2* or *MAL4*. Because of the tight genetic linkage between *MAL4* and *SIR1*, *MAL4* flanking sequences were screened for homology to

SIR1 sequences contained in plasmid pJH570 (IVY, KLAR and HICKS 1986). Significant homology was detected and the location is indicated in Figure 3. Additionally, recent genetic and physical analysis of the *BAS1* gene has shown that it is tightly linked to both *SIR1* and *MAL4* but the isolation of both *BAS1* and *SIR1* on a single genomic fragment indicates that the order is *BAS1–SIR1–MAL4* (K. ARNDT and G. FINK, personal communication). The *SIR1* containing ragment isolated by IVY, KLAR and HICKS (1986) lacks the linked *MAL4* homologous sequences and therefore appears to have been isolated from a null (*mal4⁰*) strain. Southern analysis comparing *MAL4* and *MAL2* indicates that the sequences flanking *MAL43* show some homology to those flanking *MAL23*, but homology to *SIR1* sequences in the *MAL23* flanking region, while detectable, is poor. Several restriction site polymorphisms are common to the restriction endonuclease maps of the *MAL2* and *MAL4* loci and distinguish them from the other *MAL* loci. This and the presence of *SIR1* sequence homology in the centromere proximal region of *MAL2* suggests that these loci are more closely related to each other than to *MAL1*, *MAL3* or *MAL6* and that *MAL4* is likely to be oriented with regard to the telomere and centromere in the same manner as are the other *MAL* loci.

Finally, subcloned fragments of the DNA sequences flanking GENE 3 from each of the *MAL* loci were used to scan genomic *Eco*RI and/or *Hin*dIII digested DNA to determine if these sequences are repeated in the Saccharomyces genome. Results of this analysis suggest that the sequences flanking each of the *MAL* loci seem to be unique to that *MAL* locus (with the exceptions noted above). No clearly conserved sequence was detected in the *MAL* flanking DNA sequences. Genomic Southerns revealed that two *Eco*RI fragments flanking *MAL13* contain sequences that are repeated several times throughout the genome (CHARRON and MICHELS 1988) but since these sequences are not found on the centromere proximal side of each *MAL* locus the significance of this repeated homology is not clear and may be a function of the telomere-adjacent location of the *MAL1* locus.

DISCUSSION

The *MAL* loci present in the Saccharomyces yeasts are a repeated family of polygenic loci that map to chromosome termini (MORTIMER and SCHILD 1980; MICHELS and NEEDLEMAN 1983; NEEDLEMAN and MICHELS 1983; MICHELS and NEEDLEMAN 1984). Studies on the cloned *MAL6* and *MAL1* loci demonstrated that both are complex loci and contain three genes. GENE 1 encodes the transport enzyme, maltose permease; GENE 2 encodes maltase; and GENE 3 encodes a trans-activator required for the expression of GENES 1 and 2 (NEEDLEMAN *et al.* 1984; DUBIN *et al.* 1985; CHARRON, DUBIN and MICHELS 1986; CHARRON and MICHELS 1988).

In this report we describe a comparative structural and functional analysis of the *MAL* loci and Figure 3 summarizes our results. Comparative restriction enzyme mapping and Southern analysis of the coding regions and flanking DNA sequences demonstrates that the *MAL* loci are all structurally and functionally homologous throughout an approximately 9.0-kb region containing GENEs 1, 2 and 3. Also included in this 9.0 kb homologous region is an approximately 2-kb sequence flanking GENE 2 (*Eco*RI-*Hin*dIII in Figure 3). This sequence has been shown to play no essential role in the fermentative pathway (R. A. DUBIN and C. A. MICHELS, unpublished results). Its presence at all of the *MAL* loci may be fortuitous or may be a result of the translocation process(es) occurring at telomeres.

The orientation of the *MAL1*, *MAL2*, *MAL3* and *MAL6* loci, with respect to the centromere and telomere, was shown to be CENTROMERE . . . GENE 3–GENE 1–GENE 2–[*SUC*] . . . TELOMERE. Telomere-associated *X* sequences were found flanking GENE 2 at *MAL1*, *MAL3* and *MAL6*. Additionally, the *SUC1* locus was shown to be located approximately 3 kb from *MAL12* and the *SUC3* locus was demonstrated to lie approximately 5.0 kb from *MAL32*. *MAL2* was shown by chromosomal deletion of the *HMR–MAL2* intervening sequences to have the same orientation as *MAL1*, *MAL3* and *MAL6*. Results were presented which imply that *MAL4* also is oriented as are the other *MAL* loci.

We also examined the nature of the DNA sequences on the centromere-proximal side of the locus (adjacent to GENE 3). Sequences flanking each locus are, by and large, unique to that locus. Additional homology was detected between the *MAL4–SIR1* intergenic region and *MAL2* suggesting that *MAL2* and *MAL4* are more closely related, a point that is supported by the presence of several restriction site polymorphisms common to these loci. Homology between *MAL3* and *MAL6* extends several kilobase pairs to the left of *MAL33* and *MAL63* to include the regions containing *MAL64* and *MAL34* strongly supporting the hypothesis that these loci are more closely related to each other than to the other *MAL* loci. Both *MAL1* and *MAL3* contain a linked *SUC* gene also suggesting an evolutionary relationship between the two loci. The DNA sequences between the *MAL* and *SUC* loci are different at *MAL1–SUC1* from those at *MAL3–SUC3* but these differences may reflect secondary events and not independent origins.

Clearly, the translocation of the *MAL* loci involves some form of recombination event capable of moving a large region of DNA. With the exception of *MAL3*,

the restriction endonuclease maps of the *MAL* loci shown in Figure 3 include the junction region between repeated *MAL* sequence and unique sequence DNA. Comparative Southern analysis demonstrated that this unique sequence was different for the different *MAL* loci and not repeated in the genome (except for *MAL1* flanking sequences). Thus, homologous recombination between large telomeric repeated sequences which has been suggested as a possible mechanism for *SUC* gene translocation is an unlikely mechanism for the translocation of the *MAL* loci. It is possible that a small sequence on the centromere-proximal side of the *MAL* locus could mediate a conversion of recombination event that would mobilize the *MAL* loci among telomeres. This sequence would have to be short and located on several chromosomes at multiple sites since the junctional boundaries of the different loci vary. A more likely mechanism accounting for the translocation of this polygenic family is one that involves a random break in the recipient chromosome which then heals by acquiring a new telomere from a donor chromosome bearing a telomere-linked *MAL* locus. This healing event would utilize a conversion-like process initiated by the invasion of the broken end of the recipient chromosome into a site at the centromere-proximal side of the *MAL* locus of the donor chromosome followed by the replication of all DNA sequences distal to the site of invasion on the donor chromosome. Such an event would result in the duplication of the *MAL* locus and its linked telomere onto the broken end of the recipient chromosome. Any hypothesis describing the mechanism of these translocation events could also explain the gene duplications observed at *MAL3* and *MAL6*. We suggest that these are the result of repeated translocation into the same subtelomeric region. The mechanism described above is based on previous studies which have shown that chromosome ends interact during interphase and throughout meiosis (WAGENAAR 1969; ASHLEY 1979) and the work of HABER and THORBURN (1984) which has shown that broken chromosomes have a tendency to "heal" themselves by adding on a new telomere or telomere-like structure. Events of this nature would not be a common occurrence and therefore one would not expect to find *MAL* loci at every yeast telomere.

The data described here on the dominant *MAL* loci and the data described elsewhere on the alleles of the *MAL1* locus emphasize the highly mutable nature of the subtelomeric regions of Saccharomyces chromosomes (CHARRON and MICHELS 1988). Considered as a whole, our studies of the *MAL* polygenic loci underscore the unique and indeed fluid nature of this system and perhaps of telomeres in general. The mobilization of genes among telomeres appears not to be unique to the *MAL* or *SUC* systems or to Saccharomyces. Both the *MGL* loci (encoding α-methylglucosidase) (MORTIMER and SCHILD 1980; TEN BERGE 1972) and the *HXK1* and *HXK2* genes (encoding hexokinase) (MORTIMER and SCHILD 1980; STACHELEK *et al.* 1986) are the most telomere-proximal genetic markers on their respective chromosomes and, while physical linkage to the telomere has not been demonstrated, these could also represent repeated, telomere-associated gene families. Examples of similar types of translocations are seen in other eukaryotes. The pseudoautosomal region of the human *X* and *Y* chromosomes (BUCKLE *et al.* 1985; COOKE, BROWN and RAPPOLD 1985), the VSG genes of the trypanosomes (ENGLUND, HAJDUK and MARINI 1982; VAN DER PLOEG, LIU and BORST 1984), the sex reversion factor (*Sxr*) (SINGH and JONES 1982) and the steroid sulphatase region (*STS*) of *Mus* (CRAIG and TOLLEY 1986) and the sex realizer gene of *Megaselia scalaris* (MAINX 1964; GREEN 1980) are examples. Most recently CORCORAN *et al.* (1988) describe a unique type of homologous recombination event which occurs in the subtelomeric region of *P. falciparum* which may be important in antigen diversity. The telomeric translocation of genes and gene families may represent a common mechanism of gene dispersal utilized by a variety of eukaryotic organisms thus warranting a more detailed examination of the exact process(es) involved.

We wish to thank RICHARD B. NEEDLEMAN for providing yeast strains and for critical reading of this manuscript.

We are grateful to the following individuals for providing plasmids or phage: MARIAN CARLSON for plasmid pRB117 and pRB59, JEFFREY STRATHERN for plasmid p15C, AMAR KLAR for plasmid pL85, John Ivy for plasmid pJH570, BIK-KWOON TYE and CLARENCE CHAN for plasmids YRp120, YRp131A and YRp131B, and EDWARD FRITSCH for phage EMBL3.

We would also like to thank VIRGINIA ZAKIAN for communication of results prior to publication.

This work was supported by U.S. Public Health Service Grant GM28216 from the National Institutes of Health and a grant from the Professional Staff Congress of C.U.N.Y. to C.A.M.

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Communicating editor: M. CARLSON