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The Naturally Occurring Alleles of *MAL1* in *Saccharomyces* Species Evolved by Various Mutagenic Processes Including Chromosomal Rearrangement

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Manuscript received January 19, 1988
Revised copy accepted May 26, 1988

ABSTRACT

In order for a yeast strain to ferment maltose it must contain any one of the five dominant *MAL* loci. Each dominant *MAL* locus thus far analyzed contains three genes: GENE 1, encoding maltose permease, GENE 2 encoding maltase and GENE 3 encoding a positive *trans*-acting regulatory protein. In addition to these dominant *MAL* loci, several naturally occurring, partially functional alleles of *MAL1* and *MAL3* have been identified. Here, we present genetic and molecular analysis of the three partially functional alleles of *MAL1*: the *MAL1p* allele which can express only the *MAL* activator; the *MAL1g* allele which can express both a maltose permease and maltase; and the *mal1^0* allele which can express only maltase. Based on our results, we propose that the *MAL1p*, *MAL1g* and *mal1^0* alleles evolved from the dominant *MAL1* locus by a series of rearrangements and/or deletions of this yeast telomere-associated locus as well as by other mutagenic processes of gene inactivation. One surprising finding is that the *MAL1g*-encoded maltose permease exhibits little sequence homology to the *MAL1*-encoded maltose permease though they appear to be functionally homologous.

SACCHAROMYCES strains capable of fermenting maltose carry any one of five unlinked *MAL* loci: *MAL1, MAL2, MAL3, MAL4* and *MAL6* (reviewed by BARNETT 1976). The genetic organization of the *MAL1* and *MAL6* loci has been described in detail (NEEDLEMAN *et al.* 1984; DUBIN *et al.* 1985; CHARRON, DUBIN and MICHELS 1986; Y. S. CHANG, R. A. DUBIN, C. A. MICHELS and R. B. NEEDLEMAN unpublished results). Both are complex loci consisting of three genes: GENE 1 appears to encode the maltose permease (GOLDENTHAL, COHEN and MARMUR 1983; COHEN ET AL. 1984; Y. S. CHANG, R. A. DUBIN, C. A. MICHELS and R. B. NEEDLEMAN, unpublished results); GENE 2 encodes maltase (DUBIN *et al.* 1985; HONG and MARMUR 1986); and GENE 3 encodes a *trans*-acting activator required for maltose-induced transcription of GENES 1 and 2 (CHARRON, DUBIN and MICHELS 1986; Y. S. CHANG, R. A. DUBIN, C. A. MICHELS and R. B. NEEDLEMAN, unpublished results). We refer to the three genes at the *MAL1* locus as *MAL11* (GENE 1), *MAL12* (GENE 2), and *MAL13* (GENE 3), whereas, at *MAL6*, these genes are called *MAL61* (GENE 1), *MAL62* (GENE 2) and *MAL63* (GENE 3). The gene number thus designates both the locus position and the gene function. Based on Southern analysis and restriction mapping, *MAL1* and *MAL6* have very similar nucleotide sequences throughout the region encoding the three genes (CHARRON, DUBIN and MICHELS 1986).

Both the work of NAUMOV (1971, 1972, 1976) and OSHIMA (1967) described the existence of naturally occurring partially functional *MAL* loci. Among 11 *Saccharomyces* strains isolated from the wild, NAUMOV identified two alleles of the *MAL1* locus which he referred to as *MAL1p* and *MAL1g*. Strains carrying only one of these alleles were unable to ferment maltose. In heterozygous diploids, the *MAL1p* and *MAL1g* alleles complemented and allowed for fermentation. NAUMOV also found strains that were unable to ferment maltose and could not complement the *MAL1p* or the *MAL1g* alleles. He referred to these as *mal^0* strains. NEEDLEMAN and MICHELS (1983) found *MAL1*-linked sequences homologous to *MAL6* in one *mal^0* strain, thereby identifying a fourth *MAL1* allele referred to as *mal1^0*.

The *MAL1* alleles have been functionally characterized. Strains carrying the *MAL1p* allele are complemented by cloned *MAL1* and *MAL6* sequences containing both GENE 1 and GENE 2 (that is, *MAL11* and *MAL12* of *MAL61* and *MAL62*) but not by either gene alone (NEEDLEMAN *et al.* 1984; CHARRON, DUBIN and MICHELS 1986). Thus, the *MAL1p* product is functionally homologous to the *MAL13* or *MAL63* gene product. Strains carrying the *MAL1g* allele are complemented by plasmids containing only the *MAL13* or *MAL63* gene encoding the *trans*-activator, and thus the *MAL1g* allele appears to encode both structural gene functions. Deletion/disruption analysis of the *MAL62*-homologous region of the *mal1^0* allele demonstrated that this *MAL1* allele encodes a temperature labile form of maltase (DUBIN *et al.*

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TABLE 1

***S. cerevisiae* strains used in this study**

Strain	Genotype	Source
600-1B	*MAT***a** *MAL1 SUC1 ura3-52 leu2-3,112*	CHARRON, DUBIN and MICHELS (1986)
4059	*MATα MAL1 MAL3g SUC1 leu1 ade1 ade2*	MICHELS and NEEDLEMAN (1983)
W15-7D	*MAT***a** *MAL1 leu ade*	MICHELS and NEEDLEMAN (1983)
100-1A	*MAT***a** *mal11::URA3 leu2-3,112*	CHARRON, DUBIN and MICHELS (1986)
100-1B	*MAT***a** *mal12::LEU2 ura3-52*	CHARRON, DUBIN and MICHELS (1986)
600-1BΔ13-14	*MAT***a** *mal13::URA3 leu2-3,112*	CHARRON, DUBIN and MICHELS (1986)
6-2A	*MATα* $mal1^0$ *lys2*	MICHELS and NEEDLEMAN (1983)
303-3A	*MATα* $mal1^0$ *leu2-3,112 ade1*	CHARRON, DUBIN and MICHELS (1986)
303-2B	*MAT***a** $mal1^0$ *leu2-3,112 ade*	CHARRON, DUBIN and MICHELS (1986)
328-4A	*MATα* $mal1^0$ *ura3-52 trp1 met14 ade*	NEEDLEMAN *et al.* (1984)
3-2B	*MAT***a** $mal1^0$ *lys2*	MICHELS and NEEDLEMAN (1983)
53-2C	*MATα MAL1p met*	CHARRON, DUBIN and MICHELS (1986)
236-2A	*MAT***a** *MAL1p leu2-3,112 lys2*	CHARRON, DUBIN and MICHELS (1986)
345-4A	*MAT***a** *MAL1p leu2-3,112 ura3-52 trp1 ade*	NEEDLEMAN *et al.* (1984)
JC27	*MATα MAL1g MAL3g leu2-3,112 his*	FEDEROFF *et al.* (1982)
340-2B	*MAT***a** *MAL1g ura3-52 trp1 lys2 met*	NEEDLEMAN *et al.* (1984)
340-2A	*MATα MAL1g ura3-52 ade*	R. NEEDLEMAN, Wayne State Medical School
DBY939	*MATα MALg MAL3q suc2-215 ade2 gal2*	M. CARLSON, Columbia University Medical School
DBY782	*MATα MAL1g MAL3g SUC2 ade2 gal2*	M. CARLSON, Columbia University Medical School
340-3B	*MATα MAL1g ura3-52 trp1 met14 ade*	R. NEEDLEMAN, Wayne State Medical School
628-5B	*MAT***a** *mal62::LEU2 mal12::LEU2 ura3-52 trp1 his*	DUBIN *et al.* (1985)

1985). Based on the lack of complementation by *MAL1p* or *MAL1g*, it has been assumed that this is the only *MAL* function encoded by the $mal1^0$ allele.

In this report we describe the cloning and structural analysis of the three partially functional alleles of *MAL1*. Our results suggest that these alleles were derived from the *MAL1* locus by the deletion and/or rearrangement of *MAL1* chromosomal sequences as well as by extensive sequence divergence of regions within the *MAL1* locus. Two functional alleles of the *MAL11* gene encoding maltose permease are described; one isolated from the *MAL1* dominant allele and the other from the *MAL1g* allele. Surprisingly, the two alleles exhibit little or no sequence similarity. Finally, physical linkage of the *MAL1* alleles to *SUC1*, $suc1^0$ and telomere adjacent *X* and *Y'* sequences is demonstrated.

MATERIALS AND METHODS

Strains and growth conditions: The *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. Many of the *MAL1p*, *MAL1g* and $mal1^0$ tester strains were constructed by R. NEEDLEMAN from homothallic strains kindly provided by G. NAUMOV (Institute for the Genetics of Industrial Microorganisms, Moscow). The revelant *MAL* genotype was determined using the genetic analysis described in MICHELS and NEEDLEMAN (1983) and is shown in Table 1.

Plasmids were propagated in *Escherichia coli* strain C600 or strain RR1.

Yeast strains were grown on YEP medium [1% (wt/vol) yeast extract/1% (wt/vol) peptone] plus 2% (wt/vol) glucose. Maltose fermentation is defined as the production of acid and gas in 1–3 days after inoculation and determined in 5 ml of YEP plus 2% (wt/vol) maltose medium in Durham tubes.

Preparation and analysis of DNA: Plasmid DNAs, yeast DNAs and phage DNAs were prepared as described previously (NEEDLEMAN *et al.* 1984; CHARRON, DUBIN and MICHELS 1986). Restriction endonuclease mapping, gel electrophoresis and DNA subcloning were performed using standard methods (MANIATIS, FRITSCH and SAMBROOK 1982). Southern gel transfer analysis was performed as described previously (DUBIN *et al.* 1985). Hybridizations were carried out at 65° in 0.6 M NaCl/0.06 M Na-citrate (pH 7.0)/0.2% Ficoll/0.2% polyvinylpyrrolidine/0.2% BSA/0.1% SDS/0.1 mg/ml salmon sperm DNA unless otherwise noted. DNAs were labeled with $[\alpha\text{-}^{32}P]$-dCTP (Amersham) by nick translation (RIGBY *et al.* 1977).

Yeast transformation and plasmid rescue: Yeast transformation was performed by the method of ITO *et al.* (1983) using lithium acetate. Many transformants were screened for plasmid stability and "*ARS*" function by passage through nonselective media [YEP medium plus 2% (wt/vol) glucose].

All plasmid constructs were assayed for functional *MAL* genes by the ability to complement standard *MAL1p*, *MAL1g*, or $mal1^0$ tester strains and/or *mal11Δ*, *mal12Δ*, or *mal13Δ* strains (CHARRON, DUBIN and MICHELS 1986; see Table 1). In each case, maltose fermentation was scored for approximately 50 transformants using the criteria described above.

Plasmid pY6ΔCΔH was used for site-directed integration (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1983) into the $mal1^0$ locus of strain 328-4A and the *MAL1g* locus of strain 340-2B following linearization with *Hpa*I (see Figure 1 (*) and DUBIN *et al.* 1986). Linkage of the plasmid marker (*URA3*) to the $mal1^0$ and *MAL1g* loci was determined using both physical and genetic analysis as follows. The plasmid was shown to have integrated into the appropriate *Hin*dIII fragment using Southern analysis. Each of the integrative transformants was also mated to a *MAL1 ura3-52* strain and linkage of the *URA3* marker to the nonfermenting pheno-

type determined by tetrad analysis. The *mal1^0* allele and adjacent DNA sequences were isolated from the genome of the transformant following digestion of genomic DNA with either *Hin*dIII, *Sal*I or *Bam*HI, individually, ligation of the digested DNA at low concentration, followed by selection of plasmid clones following transformation of *E. coli* strain RR1 to ampicillin resistance.

Isolation of cloned *MAL* DNA

Bacteriophage library construction and screening was as described previously (CHARRON, DUBIN and MICHELS 1986).

Cloning the *mal1^0* allele. Total genomic DNA from strain 3-2B was partially restricted with *Eco*RI, size fractionated (5.0–17.0 kb) and ligated into λgt WES.B *Eco*RI arms, packaged, and amplified as described in CHARRON, DUBIN and MICHELS (1986). The resultant library was screened using *MAL6*-derived probes (see Figure 1). Two phage clones isolated using probes pP2.2 and pD-3 (λMJC0.1 and λMJC0.2) and one clone that was isolated using pHB1 (λMJC0.3) (Figure 2) were saved for further analysis. All other clones containing smaller insert DNAs were not analyzed further. The yeast insert of phage λMJC0.2 did not overlap sequences contained in phage λMJC0.1 or λMJC0.3. Genomic linkage between these sequences was demonstrated as follows. Plasmid pM1.4B (Figure 2) was linearized with *Bgl*II and used to transform strain 328-4A to Ura$^+$. Genetic and physical analysis of one stable Ura$^+$ transformant, similar to that described for the *MAL1* locus (CHARRON, DUBIN and MICHELS 1986), showed that the plasmid had inserted at a site linked to the *mal1^0* allele of the *MAL1* locus. Briefly, integration of plasmid pM1.4B at the *mal1^0* locus was shown in the following way. The restriction map derived from the phage clones (λMJC0.1–λMJC0.3) predicted that *Sal*I and *Kpn*I sites should flank the integration site of plasmid pM1.4B in strain 328-4A::pM1.4B. Based on the restriction map of phage λMJC0.2, an approximately 6.95-kb pM1.4B homologous *Kpn*I fragment is expected. In the transformed strain this *Kpn*I fragment should increase in size to about 16.35 kb. Additionally, an approximately 9.3-kb pM1.4B homologous *Sal*I fragment is predicted if sequences contained in the nonoverlapping phage clone λMJC0.2 are actually adjacent to sequences cloned into phage λMJC0.1 and λMJC0.3. Southern analysis confirmed these predictions and in the pM1.4B transformed strain the 9.3-kb *Sal*I fragment is replaced by two *Sal*I fragments of the expected sizes (approximately 12.1 kb and 9.3 kb).

Additional *mal1^0*-linked sequences were cloned from strain 328-4A by the plasmid rescue technique of ORR-WEAVER, SZOSTAK and ROTHSTEIN (1983) using plasmid pY6ΔCΔH as described above. Results of restriction endonuclease analysis of these cloned sequences is shown in Figure 2.

Cloning the *MAL1g* allele. The *MAL1g* containing plasmid pFE52 (gift of M. CARLSON) was isolated from a total genomic DNA library of strain DBY939 (congenic to S288C) using a *suc1^0* probe as described in CARLSON, CELENZA and ENG (1985).

The *MAL1g* locus and flanking DNA sequences from strain 340-2B were also isolated using plasmid pY6ΔCΔH as described above.

Cloning *MAL1p*-linked sequences. Total genomic DNA from strain 236-2A, partially restricted with *Eco*RI (6.0–20.0 kb), was ligated into the lambda phage vector EMBL3. The resulting library was screened with the *MAL6*-derived probe pH-2 (Figure 1). Phage λMJC1p.1 was analyzed in detail. Genomic linkage of the *Eco*RI fragments contained in this phage was confirmed using a method similar to the one described above for phage λMJC0.2 carrying inserts from the *mal1^0* locus. Here, in the analysis of *MAL1p*, the plasmid pM1.4B was integrated into the *MAL1p* strain 345-4A by linearization with *Bgl*II.

Cloning of a putative *mal* pseudogene. Total genomic DNA from strain W15-7D was digested with *Bam*HI, ligated into EMBL3 *Bam*HI arms and the resultant library screened with the *MAL6*-derived probes pD-1 and pP2.2 (Figure 1). Two identical clones were isolated (λMJC1.7 and λMJC1.8). λMJC1.7 was further characterized.

RESULTS

Unlike most cloned yeast sequences which encode proteins, sequences similar to the sequence of the three genes of the *MAL6* or *MAL1* loci are not unique in the yeast genome. When *MAL* sequences are used in Southern analysis to probe strains carrying 1 or 2 functional *MAL* loci, several more than 1 or 2 hybridizing fragments of unknown genomic location and function are seen. The presence of these repeated copies complicates efforts to isolate the DNA fragments encoding the *MAL* loci or their alleles since probing a library for related sequences will lead to the isolation of a variety of different fragments derived from several genomic sites. For this reason, the results described here utilize both physical and genetic methods to demonstrate unequivocably that the desired locus has in fact been isolated.

Cloning of the *mal1^0* allele: The *mal1^0* allele of strain 3-2B was isolated by plaque hybridization to a λgtWES.B library using *MAL6* probes as described in MATERIALS AND METHODS. Three phage clones were isolated and restriction maps of their yeast inserts are shown in Figure 2. Phage λMJC0.1 and λMJC0.3 contain overlapping yeast inserts and, based on restriction endonuclease mapping and Southern analysis, both exhibit extensive, but not complete, homology to the *MAL1* locus (described in greater detail below). The yeast insert in phage λMJC0.2 does not overlap those of the other phage isolates but restriction endonuclease mapping and Southern analysis using *MAL1* probes from the *MAL13* flanking sequences (fragments a, b and c shown in Figure 2) indicate that the λMJC0.2 insert and *MAL1* are highly homologous in this region. To demonstrate that the nonoverlapping sequences contained in phage clones λMJC0.2 and λMJC0.3 are linked in the genome as they are drawn in Figure 2, continuity between these fragments had to be established. This was accomplished by targeting the integration of plasmid pM1.4B to the *Bgl*II site indicated in Figure 2 (*) and showing by Southern analysis that this integration had occurred within a *Kpn*I fragment of the size predicted by the restriction maps of the phage λMJC0.2 and λMJC0.3 (see MATERIALS AND METHODS for details). A composite restriction map of all three phage clones is presented in Figure 2. Genetic analysis of the strain carrying the integrated plasmid pM1.4B also demonstrated linkage of the plasmid to *mal1^0* indicating that

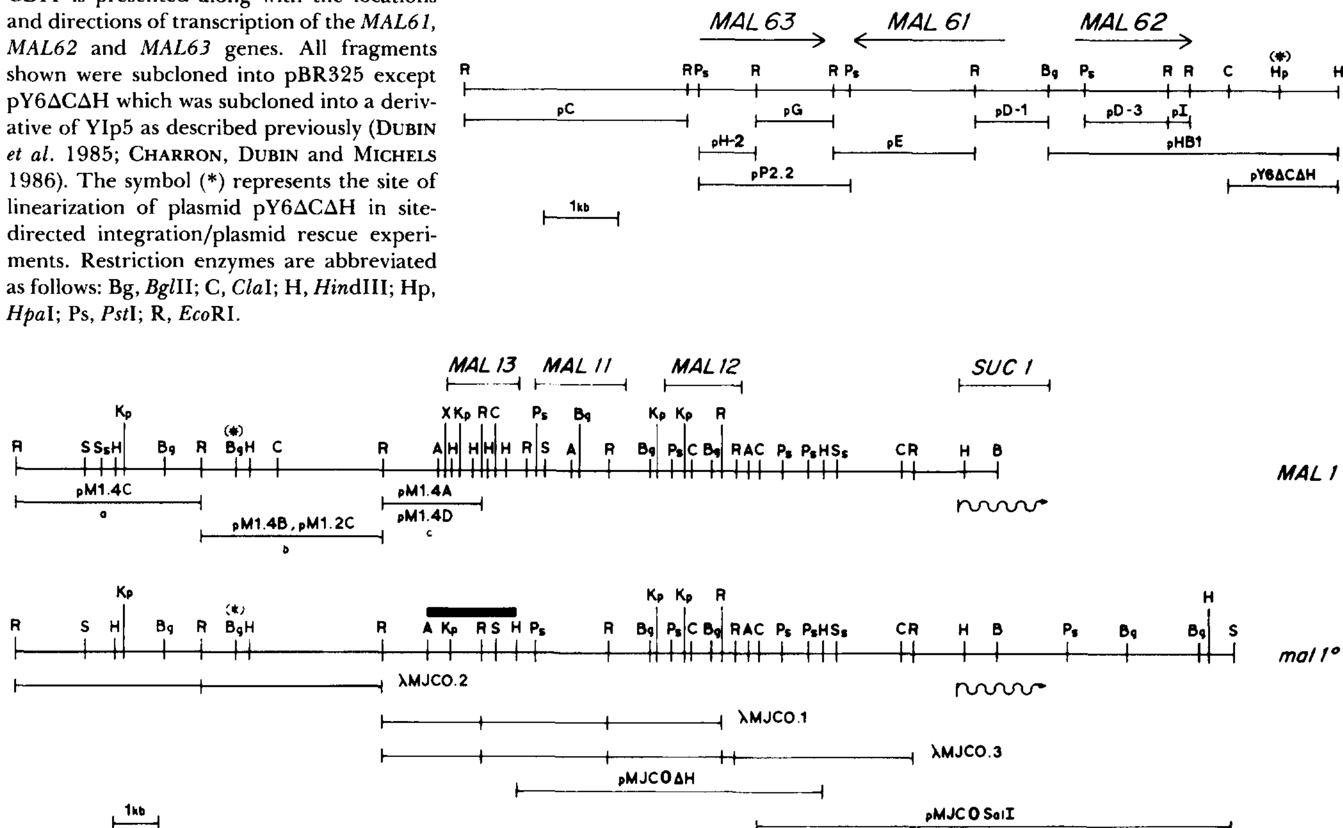

FIGURE 1.—Subclones of the (wild-type) *MAL6* locus from strain CB11. A partial restriction map of the *MAL6* locus of strain CB11 is presented along with the locations and directions of transcription of the *MAL61*, *MAL62* and *MAL63* genes. All fragments shown were subcloned into pBR325 except pY6ΔCΔH which was subcloned into a derivative of YIp5 as described previously (DUBIN *et al.* 1985; CHARRON, DUBIN and MICHELS 1986). The symbol (*) represents the site of linearization of plasmid pY6ΔCΔH in site-directed integration/plasmid rescue experiments. Restriction enzymes are abbreviated as follows: Bg, *Bgl*II; C, *Cla*I; H, *Hin*dIII; Hp, *Hpa*I; Ps, *Pst*I; R, *Eco*RI.

FIGURE 2.—Restriction endonuclease maps of the *MAL1* and *mal1*^{0} loci. The complete restriction map of the *MAL1* locus is shown at the top of the figure. The approximate size and location of the *MAL11*, *MAL12* and *MAL13* genes are indicated. Regions of the *MAL1* locus were subcloned into pBR325 to form plasmids pM1.2C and pM1.4D or into YIp5 to form plasmids pM1.4A, pM1.4B and pM1.4C (CHARRON, DUBIN and MICHELS 1986). The restriction map of the 27.3-kb sequence of DNA containing the *mal1*^{0} locus is shown below that of the *MAL1* locus. Overlapping yeast inserts contained in the phage isolates λMJC0.1 through λMJC0.3 and the plasmids pMJC0ΔH and pMJC0SalI (see MATERIALS AND METHODS) are shown at the bottom of the figure. The symbol (*) represents the site of integration of plasmid pM1.4B into strain 328-4A. The solid horizontal bar represents a region of poor homology to *MAL13*-derived probes. The region showing homology to the *SUC2* gene probes pRB117 and pRB59 and the direction of transcription of the *SUC1* gene are indicated by the wavy line. Restriction enzymes are abbreviated as in Figure 1 with the following additions: A, *Ava*I; B, *Bam*HI; Kp, *Kpn*I; S, *Sal*I; Ss, *Sst*I; X, *Xho*I.

these sequences are derived from this locus.

Additional physical analysis of the cloned fragments support our conclusion that these sequences contain the *mal1*^{0} locus of strain 3-2B. Phage λMJC0.3 contains a 7.0 kb *Hin*dIII fragment homologous to the *MAL6* plasmid pD-1. Genomic Southern analysis of strain 3-2B also shows a single 7.0-kb *Hin*dIII fragment homologous to this probe. Previous studies have shown linkage between the *mal1*^{0} allele and a 7.0 kb *Hin*dIII fragment having homology to pD-1 (NEEDLEMAN and MICHELS 1983; MICHELS and NEEDLEMAN 1983, 1984).

Complementation activity of plasmid subclones derived from phage λMJC0.3 verifies our conclusion that this phage clone contains sequences from the *mal1*^{0} allele. We constructed plasmids pM0.3A and pM0.3B containing the 7.0-kb pD-1 homologous *Hin*dIII fragment of phage λMJC0.3 (Figure 2) in YIp5 and YEp13, respectively. Based on our previous functional analysis of strains carrying the *mal1*^{0} allele, it is expected that these sequences encode only maltase (DUBIN *et al.* 1985). When plasmid pM0.3A is transformed into strain 100-1B, a maltose nonfermenting strain that carries a deletion/disruption of *MAL12* (encoding maltase), complementation is observed. Similarly, pM0.3A complements the *MAL62/MAL12* double disruption strain 628-5B. The absence of any other *MAL* complementing activity on plasmids pM0.3A and pM0.3B is seen as follows. Both plasmids were transformed independently into *MAL1p* (236-2A and 345-4A) tester strains which express only functional activator. No complementation was observed indicating that the plasmids do not encode functional maltose permease. These plasmids were also transformed independently into *MAL1g* (340-2B and JC27) and *mal1*^{0} (303-3A and 328-4A) tester

strains and, as expected, complementation was not observed. Taken together, the functional evidence described above clearly demonstrates that the clones isolated are derived from the *mal1⁰* allele.

The *mal1⁰* allele of strain 328-4A, along with flanking DNA, was isolated through a plasmid rescue technique as described in the MATERIALS AND METHODS. Plasmids pMJC0ΔH and pMJC0SalI were obtained (Figure 2). Both strains 3-2B and 328-4A were derived from the same *mal1⁰* strain of NAUMOV (*Saccharomyces chevalieri* strain 407) by crosses to laboratory strains carrying the *MAL1g* allele of *MAL1* and therefore their *mal1⁰* loci are expected to be identical. The restriction endonuclease map of the yeast insert in plasmid pMJC0ΔH is identical to that of λMJC0.3 and plasmid pMJC0ΔH complements the *MAL62/MAL12* double disruption strain 628-5B but not the *MAL1p, MAL1g* and *mal1⁰* tester strains supporting our contention that 3-2B and 328-4A contain identical *mal1⁰* alleles. Therefore, the restriction endonuclease map of the *mal1⁰* allele diagrammed in Figure 2 is a composite of the 3-2B and 328-4A clones.

Figure 2 compares the structure of the *MAL1* and *mal1⁰* loci. Several striking features become clear. The overall size and restriction map of both loci are largely identical and only a few polymorphisms are seen. Southern analysis of the cloned *mal1⁰* sequences using probes derived from *MAL1* (fragments a, b and c of Figure 2), from *MAL6* (the *MAL61-62-63* coding regions shown in Figure 1) and from the coding region of *SUC2* demonstrates that the loci are highly homologous with the exception of an approximately 2 kb region shown in Figure 2 as a solid overbar. Similarity between the *mal1⁰* sequences in this region and sequences derived from the MAL activator gene is poor as evidenced by weak hybridization between *MAL63* plasmid subclones and phage DNA from λMJC0.1 and λMJC0.3. The *MAL1* and *mal1⁰* sequences diverge near the *Ava*I site in fragment c (Figure 2). Several restriction site polymorphisms are seen between the *MAL1* and *mal1⁰* alleles in this region. None the less, the overall size of the region remains the same and the *Eco*RI restriction map, with the exception of one lost site, has been retained. Therefore, we consider it unlikely that a rearrangement involving this region led to the loss of function of the *MAL13* gene and suggest that extensive sequence divergence is responsible.

Both the *MAL1* and *mal1⁰* cloned loci have a linked *SUC* gene. The *MAL1* strain used to clone the *MAL1* locus is a sucrose fermenter and contains the *SUC1* locus (CHARRON, DUBIN and MICHELS 1986). The *mal1⁰* strains used in this study also ferment sucrose. As diagrammed in Figure 2, *SUC*-homologous sequences are found flanking both alleles and the restriction map of plasmid pMJC0SalI closely resembles that of the *MAL1-SUC1* locus intergenic region (CARLSON, CELENZA and ENG 1985). The *mal1⁰* locus therefore also appears to be linked to the telomere-adjacent *SUC1* locus.

One unusual feature of both the *MAL1* and the *mal1⁰* loci not shown in Figure 2 is worth pointing out. When probes derived from the *MAL61-MAL62* region (pD-1 and pD-3, Figure 1) are used to probe the *mal1⁰* phage clones, weak hybridization to fragments c and b of λMJC0.1 and λMJC0.2 is seen in addition to the expected strong hybridization to the 2.5-kb *Eco*RI fragment of λMJC0.1 and λMJC0.3. Results obtained here within the *mal1⁰* allele have been noted previously in the *MAL1* locus itself (CHARRON, DUBIN and MICHELS 1986). The exact meaning of this apparent repeated sequence is not known.

Based on the functional and physical analyses presented above, the genes present at the complex *mal1⁰* locus may be represented as *mal11 MAL12 mal13*.

Analysis of *MAL1g* clones: Physical analysis of *MAL1g* strains revealed that a 10.7-kb *Hin*dIII fragment, homologous to the *MAL6*-derived probe, pD-1, is linked to the *MAL1g* locus (MICHELS and NEEDLEMAN 1983, 1984; NEEDLEMAN and MICHELS 1983). This 10.7-kb *Hin*dIII fragment is found within plasmid pFE52 (Figure 3). Briefly, plasmid pFE52 was isolated by homology to *suc1⁰* probes from a YEp24/*Sau*3A partial library of a strain DBY939 (congenic to strain S288C) (CARLSON, CELENZA and ENG 1985). Plasmid pFE52 contains *MALg* function (GENE 1 and GENE 2) as evidenced by its ability to complement a *MAL1p* strain (345-4A) encoding only the activator, but not *MAL1g* or *mal1⁰* strains (340-2B and 328-4A, respectively) which lack the activator function. The GENE 1 and GENE 2 complementing activity of pFE52 was localized within the yeast insert by plasmid subcloning and Figure 3 summarizes the results (see legend for full details regarding strains used). The *MALg* complementing activity of pFE52 is localized to the 9.2-kb *Bam*HI/*Hin*dIII fragment which is able to complement strains lacking both GENE 1 and GENE 2 function (strains 345-4A and 236-2A). Deletion of the 1.5-kb *Bgl*II fragment disrupts the ability to complement strains lacking GENE 2 function (plasmid pM1gAΔBglII in the *mal12Δ* strain, 100-1B) but not the ability to complement strains lacking GENE 1 function (plasmid pM1gEΔBglII in the *mal11Δ* strain, 100-1A). Therefore, maltose permease is encoded by the 5.4 kb *Bam*HI/*Bgl*II fragment and maltase is encoded by the sequences near the 1.5-kb *Bgl*II fragment.

The location of the structural genes present at the *MAL1g* allele is similar to that found at the dominant *MAL1* locus but significant divergence between the restriction maps of these two cloned regions is quite evident from Figure 3. To determine the extent of

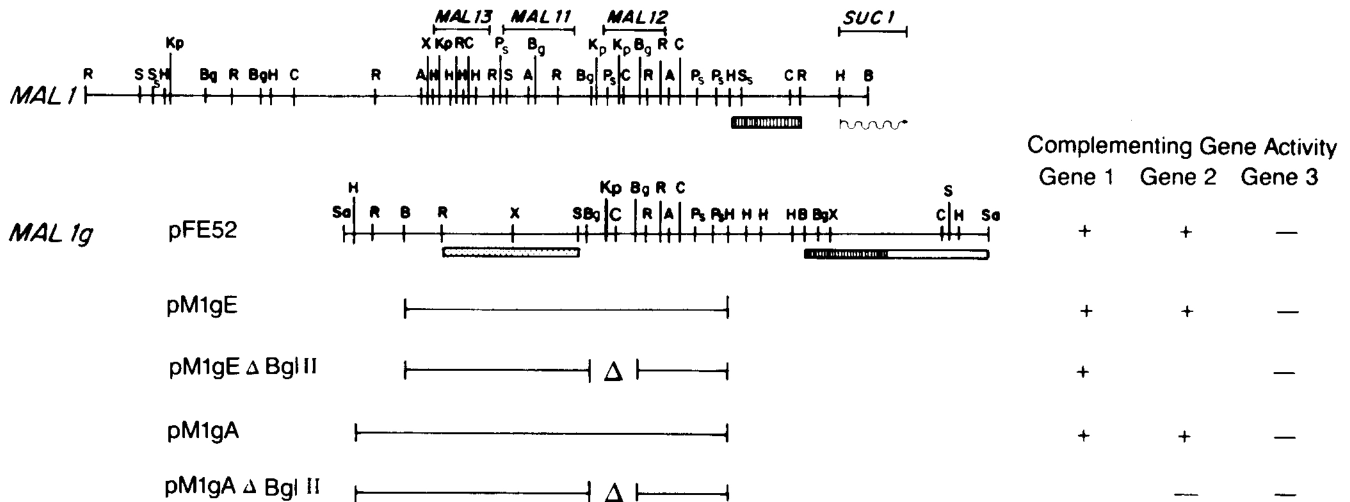

FIGURE 3.—Structural and functional comparison of the *MAL1* and *MAL1g* loci. The complete restriction map of the *MAL1* locus is shown at the top of the figure (CHARRON, DUBIN and MICHELS 1986). Beneath this is the restriction map of the yeast insert of plasmid pFE52 containing the *MAL1g* locus and plasmid subclones of this region. The vector of plasmid pFE52 is YEp24, of plasmids pM1gE and pM1gEΔBglII is YEp13 and of plasmid pM1gAΔBglII is YIp5. The 10.7 kb *Hin*dIII fragment of pFE52 has a weak ARS activity and therefore plasmid pM1gAΔBglII is maintained as an episome. The strains used for the complementation tests were 100-1A, 100-1B, JC27, 345-4A, 236-2A, 303-3A, 328-4A, and 340-2B (see Table 1 for genotypes). The region of homology between the *MAL1* and *MAL1g* loci is indicated by the vertical dashed lines. The stippled bar indicates the region of poor homology to *MAL61*-derived probes (maltose permease gene of *MAL6*). The ability of each plasmid to complement *MAL11, MAL12* or *MAL13* gene functions is indicated. Restriction enzymes, and other symbols, are as described in Figure 2.

the sequence similarity between *MAL1g* and the other cloned *MAL* loci, Southern analysis using probes derived from both the *MAL1* and *MAL6* loci was performed. The results are summarized in Figure 3. Vertical dotted lines mark the boundaries of the *MAL1–MAL1g* homologous region which is limited to the maltase structural gene and approximately 2.0 kb of DNA immediately to the right of *MAL12*. Sequence divergence in the region far to the right of *MAL12* is consistent with the fact that the *MAL1* strain used in this study carries the *SUC1* locus (CHARRON, DUBIN and MICHELS 1986), whereas the *MAL1g* strain DBY939 carries the *suc1^0* allele (CARLSON, CELENZA and ENG 1985). Analysis of *suc1^0* and other *suc^0* alleles, except *suc2^0*, has shown that these alleles lack *SUC*-homologous sequences and several kilobase pairs of flanking DNA (CARLSON, CELENZA and ENG 1985).

Sequence divergence in the region to the left of the *MAL12* gene is surprising. Sequences homologous to probes derived from GENE 3 (plasmids pH-2 and pG, Figure 1) are completely absent from plasmid pFE52 and no sequence similarity is found between this region of plasmid pFE52 and *MAL1* flanking sequences (fragments a, b and c). Even more remarkable is the finding that little or no sequence similarity is evident between the GENE 1 probe (plasmid pE, Figure 1) and the region of the *MAL1g* locus which was shown to contain functional permease. (This region is indicated by the stipled bar in Figures 3 and 4.) Thus, the sequence of the alleles is unrelated over this region yet both sequences encode functional maltose permeases. We refer to the gene encoding the maltose permease of the dominant *MAL1* locus as the *MAL11-1* allele and the gene encoding the maltose permease of the partially functional *MAL1g* locus as the *MAL11-2* allele.

The *MAL1g* allele from strain 340-2B was also isolated using the integrating plasmid pY6ΔCΔH as described in MATERIALS AND METHODS. Both restriction endonuclease analysis and Southern analysis, gave results identical to those obtained for plasmid pFE52.

In summary, the genes encoded by the *MAL1g* locus may be represented as, *MAL11-2 MAL12 mal13Δ*.

Structural analysis of the *MAL1p* allele: Sequences linked to the *MAL1p* allele of strain 236-2A were isolated in phage λMJC1p.1 which was selected using the *MAL63*-derived probe pH (Figure 1) as described in MATERIALS AND METHODS. Restriction mapping and Southern analysis using plasmid probes containing fragments a, b and c derived from the sequences flanking *MAL13* of the *MAL1* locus (see Figure 2 for probes) clearly demonstrated homology between the entire yeast insert contained in λMJC1p.1 and the equivalent region of the *MAL1* locus (summarized in Figure 4). The only observed difference, other than a few restriction endonuclease polymorphisms, is a small insert (approximately 100 bp) into the *MAL1p* locus located within the 4.1-kb *Eco*RI fragment at the extreme left-hand end of the sequences as drawn in Figure 4. Further demonstration that the insert found in λMJC1p.1 is in fact linked to *MAL1p* was obtained by targeting the integration of plasmid pM1.4B into

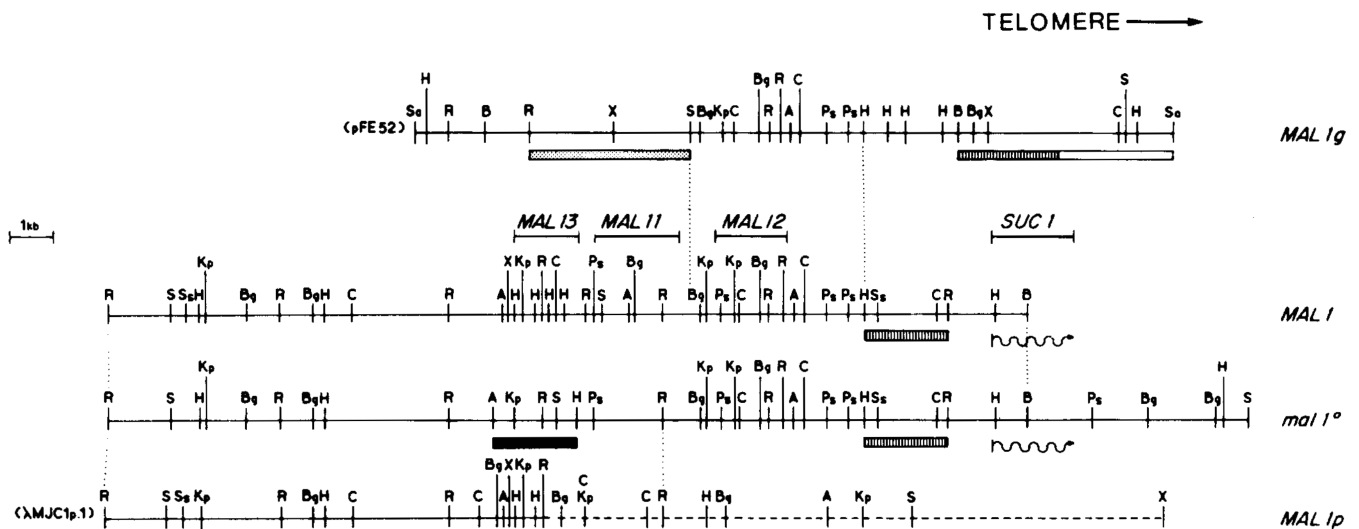

FIGURE 4.—Structural comparison of the *MAL1* alleles. The restriction endonuclease map of the *MAL1*, *mal1*^{0}, *MAL1p* and *MAL1g* alleles are diagrammed. The approximate size and location of the *MAL11*, *MAL12* and *MAL13* genes are shown. Horizontal solid lines indicate cloned sequences and horizontal dashed lines indicate results obtained from genomic Southern analysis. The boundaries of homology between *MAL1* and the three partially functional alleles are indicated by vertical dotted lines. The solid bar designates poor homology to probes derived from the *trans*-acting activator. The stippled bar indicates the region of poor homology to *MAL61*-derived probes (maltose permease gene of *MAL6*). Homology to telomere-derived *X* sequences (YRp120 and YRp131A) are diagrammed with vertical hatched bars and the *Y'* sequences (YRp131B) with open bars. Homology to *SUC1* (pRB117 and pRB59) is also indicated. Recognition sites for restriction endonucleases are abbreviated as in Figures 1 and 2.

the genome of the *MAL1p* strain 236-2A. This was performed by linearization at the *Bgl*II site indicated in Figure 2 (*). Linkage was shown between the *URA3* gene of the integrated plasmid and the *MAL1p* locus and by demonstrating that the plasmid had inserted within the *Kpn*I fragment of the size predicted by the restriction map of λMJC1p.1. (For details, see MATERIALS AND METHODS.)

Only the sequences from the left-end of the regulatory gene, GENE 3, are present in λMJC1p.1 (Figure 3). We therefore attempted to clone the remainder of the *MAL1p* allele using a variety of methods but, despite intensive efforts, the cloning of this *MAL1* allele could not be completed. The reasons for our lack of success are not apparent, and we can only suggest that the cloning of the remainder of the *MAL1p* locus requires very different cloning strategies than those utilized in this study.

The restriction map of the remainder of the *MAL1p* locus, shown in Figure 4 as a dashed line, was derived from Southern analysis of total genomic DNA from strain 236-2A using probes spanning the *MAL6* and *MAL1* loci (Figures 1 and 2). Only homology to the plasmid probes pE and pG derived from the GENE 2–GENE 3 region of *MAL6* could clearly be demonstrated. The vertical dotted lines shown in Figure 4 indicate the proposed extent of homology between the *MAL1* and *MAL1p* loci.

Based on these results, the genes of the *MAL1p* locus may be represented as *mal11 mal12*Δ *MAL13*.

Comparative Southern analysis of the flanking DNA sequences of the *MAL1* alleles. *MAL1* and *SUC1* are located at the distal end of the right arm of chromosome *VII* (CELENZA and CARLSON 1985; CARLSON, CELENZA and ENG 1985). We wished to determine the relationship between the telomere-associated repeated sequences, *X*, $(C_{1-3}A)_n$ and *Y'* and the *MAL1* alleles (WALMSLEY, SZOSTAK and PETES 1984). Homology to *X* sequences was determined using plasmid YRp120 (CHAN and TYE 1983) and is detected in the cloned *MAL1*, *mal1*^{0} and *MAL1g* flanking DNA sequences (see Figure 4, shown as vertically hatched bars). *Y'* sequences, homologous to pYR131B (CHAN and TYE 1983), were found only in the sequences flanking *MAL1g* (see Figure 4, shown as an open bar). Poly[dG-T]·Poly[dC-A] was used to detect $(C_{1-3}A)_n$ repeat sequences flanking the cloned *MAL1* alleles. Homology was only detected on the right-hand end of the loci as drawn in Figure 4, but was not precisely localized. Each of these characteristically telomere-associated sequences is found only to one side of the *MAL1* alleles and, based on the location of the *SUC1* and *suc1*^{0} loci, this must represent the telomere proximal side. Thus, the *MAL1*, *MAL1g* and *mal1*^{0} loci are each oriented in the same way with regard to the centromere and telomere of chromosome *VII*. While a similar analysis of the *MAL1p* locus was not possible, we propose that this allele has the same orientation as the other *MAL1* alleles. This conclusion is based on the excellent homology between the cloned sequences adjacent to the *MAL13* gene of the *MAL1p* locus and several kilobase pairs of DNA from the centromere

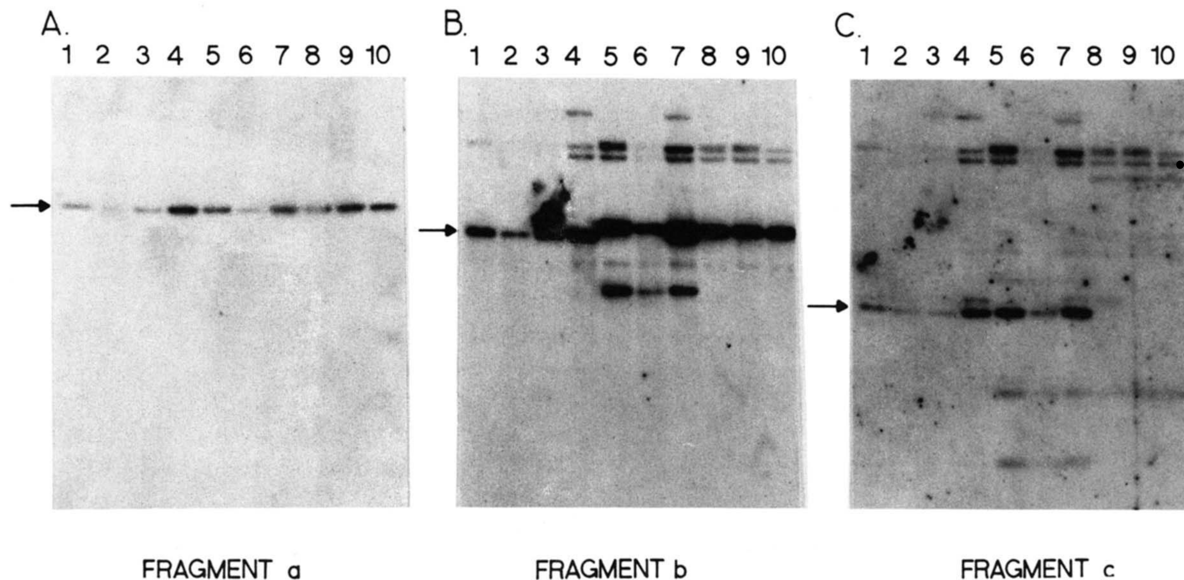

FIGURE 5.—Southern analysis of *MAL1, mal1⁰, MAL1p* and *MAL1g* strains probed with *MAL1*-derived centromere-proximal flanking DNA sequences. Total genomic DNA from several strains carrying *MAL1* alleles was digested with *Eco*RI and subjected to Southern analysis. The three panels differ with regard to the hybridization probe as follows: panel A was probed with a plasmid carrying fragment a (plasmid pM1.4C), panel B was probed with a plasmid carrying fragment b (plasmid pM1.2C), and panel C was probed with a plasmid carrying fragment c (plasmid pM1.4A). Fragments a, b and c are derived from the centromere proximal side of *MAL1* (see Figure 2). The arrows indicate the size of the fragment used in the hybridizations. Lane 1, 600-1B (*MAL1*); lane 2, 328-4A (*mal1⁰*); lane 3, 3-2B (*mal1⁰*); lane 4, 6-2A (*mal1⁰*); lane 5, 236-2A (*MAL1p*); lane 6, 345-4A (*MAL1p*); lane 7, 53-2C (*MAL1p*); lane 8, DBY782 (*MAL1g MAL3g*); lane 9, 340-3B (*MAL1g*) and lane 10, 340-2B (*MAL1g*).

proximal DNA flanking the *MAL1* and *mal1⁰* alleles (see below).

It was also of interest to know whether any of the centromere-proximal flanking sequences were present at multiple genomic locations. The results of Southern blots performed on genomic *Eco*RI digested DNA from *MAL1, mal1⁰, MAL1p* and *MAL1g* strains probed with fragments a, b and c derived from the *MAL1* locus (Figure 2) are shown in Figure 5. Fragment a is unique in the genome. In contrast, fragments b and c are repeated several times in all of the strains analyzed (2–9 *Eco*RI fragments depending upon the strain analyzed). Hybridization to all but one or two of the fragments is dramatically reduced when hybridizations are carried out at increased stringency (0.4 M NaCl) (data not shown). Thus, these sequences are imperfectly repeated in the genome of these strains. The function of this moderately repeated element, if any, is unclear.

Cloning putative *mal* pseudogene sequences: Upon screening the *MAL1* library (W15-7D:EMBL3) with probes pP2.2 and pD-1 (Figure 1) phage λMJC1.7 was isolated (MATERIALS AND METHODS). Preliminary restriction endonuclease mapping of the 16.0-kb *Bam*HI insert suggested that something other than the *MAL1* locus had been isolated. Southern gel transfer analysis (data not shown) indicates that the yeast insert hybridizes to the probe pD-1. When the *MAL61-MAL62* intergenic plasmid pD-1 is used to probe *Bam*HI digested *MAL1* genomic DNA, a strongly hybridizing approximately 24.0-kb fragment is detected as well as a weakly hybridizing 16.0-kb fragment. The 24.0-kb *Bam*HI fragment corresponds to the *MAL1* locus. The 16.0-kb fragment present in λMJC1.7 corresponds to the smaller *Bam*HI fragment which only poorly hybridizes to plasmid pD-1.

Phage λMJC1.7 was further analyzed using other *MAL6*- and *MAL1*-derived sequences and the results show some sequence similarities to GENE 2 sequences encoding maltase and to sequences flanking the right side of GENE 2. Weak hybridization to *X* sequences (YRp120) was also detectable within the insert suggesting that this sequence, like the *MAL* loci, may also be located at or near a telomere.

This fragment does not encode functional maltase. The insert DNA of λMJC1.7 was subcloned into YIp5 and tested for its ability to complement the defect in the *mal12Δ* strain 100-1B. The phenotype of the resulting transformant remained maltose nonfermenting. This result is consistent with the genetic analysis of strain W15-7D showing that it contains only one functional maltase gene, that which is present at the *MAL1* locus (R. NEEDLEMAN, personal communication). Whether this fragment contains a nonfunctional copy of the maltase gene whose sequence has undergone a great deal of alteration or simply encodes a gene which has some sequence similarity to *MAL12* but whose function and origin is unrelated to maltose fermentation is undetermined.

TABLE 2

Summary of the genotypes of the *MAL1* complex locus and its partially functional alleles

MAL1 Allele	Genotype		
MAL1	*MAL11-1*	*MAL12*	*MAL13*
MAL1g	*MAL11-2*	*MAL12*	*mal13*Δ
MAL1p	*mal11*	*mal12*Δ	*MAL13*
mal1^0	*mal11*	*MAL12*	*mal13*

DISCUSSION

In a previous report we describe the cloning and functional analysis of the *MAL1* locus (CHARRON, DUBIN and MICHELS 1986). Results of this study have shown that *MAL1*, like *MAL6*, contains three genes (*MAL11*, *MAL12* and *MAL13*) within an approximately 9.0-kb region. These *MAL1* genes are both structurally and functionally homologous to their *MAL6* counterparts (*MAL61*, *MAL62* and *MAL63*). Additionally, the telomere-associated *SUC1* locus was found to be located 5.0 kb to the right of *MAL12*. Here we extend our study of the *MAL1* locus to include all four alleles of *MAL1* (*MAL1*, *mal1*^{0}, *MAL1p* and *MAL1g*). Through the combined cloning and physical analysis of the three partially functional alleles of *MAL1* (*mal1*^{0}, *MAL1p* and *MAL1g*) we demonstrate that the *MAL1* alleles appear to be derived from the *MAL1* locus by the rearrangements and/or deletion of portions of the *MAL1* locus as well as by the accumulation of point mutations within coding regions. This study allows us to specify the status of the *MAL* genes encoded by each of the partially functional alleles using our previously established nomenclature and this is summarized in Table 2.

Figure 4 compares the structure of the four *MAL1* alleles. Several features are noteworthy. The right-hand side of the *MAL12* genes of *MAL1* and *mal1*^{0} are flanked by *X* sequences and *SUC1*, and the right-hand side of the *MAL12* gene of *MAL1g* is flanked by *X*, *Y'* and *suc1*^{0} sequences. The presence and location of these telomere-associated sequences clearly indicates that each of these loci is oriented in the same direction with regard to the centromere and telomere and this is indicated in Figure 4. Due to the repeated nature of telomere-associated sequences it was impossible to determine linkage of *MAL1p* flanking DNA sequences to either *X* or *Y'* by Southern gel transfer of genomic DNA but, based on the extensive homology between the cloned *MAL1p* sequences and the sequences to the left of the *MAL13* gene, we suggest that this allele is similarly oriented.

Loss of function of a particular gene in each of the partially functional alleles appears to have occurred by one of two mechanisms: the accumulation of point mutations and/or genomic rearrangement. Extensive sequence and restriction map homology to the *MAL11* gene of the *MAL1* locus is found within the *mal1*^{0} locus yet function is lost indicating that a discrete number of point mutations has inactivated the gene. *MAL13* gene function also has been lost at the *mal1*^{0} locus but here very little sequence similarity to the *MAL13* gene remains. This poor sequence homology could indicate that a large number of point mutations have accumulated within this gene over time but an alternate mechanism can be proposed. As mentioned above, repeated copies of the *MAL* coding sequences having unknown function are present in all strains. We suggest that a gene conversion-like event involving the *MAL1* locus and one of these repeated sequences having some sequence similarity to *MAL13* could have resulted in the loss of function of the *MAL13* gene of the *mal1*^{0} allele and to the formation of this weakly hybridizing region. Such events have been documented previously in yeast (ERNST, STEWART and SHERMAN 1981).

Chromosomal rearrangement has clearly led to the formation of the *MAL1g* allele. Linked *MAL13* sequences are entirely lost from the cloned fragment. More remarkably, the fragment shows little or no sequence similarity to the *MAL11* gene (encoding maltose permease) at the *MAL1* locus yet a functional maltose permease is encoded. It appears that a large deletion/rearrangement has occurred which has removed the *MAL11* and *MAL13* genes from their normal genomic position and replaced them with sequences derived from elsewhere in the genome. This new linked sequence fortuitously encodes a protein capable of transporting maltose and the rearrangement has positioned it so that its expression is under the control of maltose and the maltose activator. Perhaps this different maltose permease gene is derived from the structural gene of another sugar transport system, the most likely candidates being alpha-methylglucoside permease and maltotriose permease (TEN BERGE 1972). Southern analysis using probes derived from this new maltose permease indicate that the sequence is present in strains carrying the dominant *MAL1* allele. However, since deletion of the *MAL11* gene in these strains leads to a nonfermentor, this new maltose permease clearly does not function as a maltose transport protein in these strains most probably because the gene is not normally induced by maltose. Because of the repeated nature of the *MAL11* and *MAL13* sequences, we are unable to determine if they have been deleted from the genome of *MAL1g* strains or simply located at a different genomic position. Since we have not isolated the entire *MAL1p* locus, we are unable to draw any strong conclusions regarding the alterations at this site but our results indicate that the *MAL11*-homologous sequences are present at this locus but have been inactivated by point mutation(s) and that the *MAL12* gene

has been lost from this site by deletion or rearrangement.

It has been shown that extensive structural polymorphisms exist in telomeres of different strains, as well as within the same strain of *S. cerevisiae* and these regions undergo frequent rearrangements (HOROWITZ, THORBURN and HABER 1984; BUTTON and ASTELL 1986; ZAKIAN, BLANTON and WETZEL 1986). The highly polymorphic nature of the *MAL1* alleles is quite evident in the results of these analyses. The polymorphisms are structural as well as functional. Perhaps the telomere-adjacent location of the *MAL1* locus has contributed to the apparent mutability of this locus which has resulted in the formation of three partially functional alleles (*MAL1p, MAL1g* and *mal1*^{0}). The *MAL1* locus is the only *MAL* locus known to have four alleles. The telomere-associated *MAL3* locus has two known alleles: the fully functional *MAL3* allele and the partially functional *MAL3g* allele. A complete molecular analysis of the *MAL3* alleles will be presented elsewhere (M. J. CHARRON and C. A. MICHELS, unpublished results). The other *MAL* loci (*MAL2, MAL4* and *MAL6*) are also linked to telomeres, however, partially functional alleles of these loci have not been found in our laboratory strains (NEEDLEMAN and MICHELS 1983; MICHELS and NEEDLEMAN 1984). Perhaps genetic and physical analysis of strains isolated from the wild is needed to determine if *MAL2, MAL4* and/or *MAL6* have partially functional alleles similar to those linked to *MAL1* and *MAL3*.

All yeast strains that have been analyzed genetically have been shown to contain one of the alleles of the *MAL1* locus suggesting that *MAL1* may be the progenitor *MAL* locus. To examine this possibility the copy number of DNA sequences flanking the centromere-proximal side of the *MAL1* locus were analyzed. It has been proposed that polygenic families such as the *SUC* and *MAL* loci were produced by inter-chromosomal homologous recombination events involving sequences found at several (or all) telomeres. Fragments b and c (Figure 2) were shown to be repeated several times in most *MAL1* strains analyzed (Figure 5) and therefore are candidates for mediating such recombination events. DNA sequences flanking the other *MAL* loci have been cloned and show no homology to these fragments suggesting that fragments b and c were not influential in the mobilization of the *MAL* loci (M. J. CHARRON and C. A. MICHELS, unpublished results). Our results do not exclude the possibilities that a small sequence in fragment b and/or c is common to all *MAL* loci and that the probes used were just too large to detect this small repeat, or that sequence differences could have arisen subsequent to the translocation event.

It is interesting to note that in most yeast strains analyzed hybridization to probes derived from GENE 1, GENE 2 and GENE 3 is observed at several genomic locations outside of the approximately 9.0-kb boundary of the *MAL* locus. In the course of screening libraries with *MAL*-gene probes it is therefore expected that several different unlinked fragments will be isolated. We describe the cloning and partial functional analysis of one such sequence (λMJC1.7) from a *MAL1* library. The insert in λMJC1.7 shows poor but significant homology to probe pD-1. Our results do not allow us to decide whether this homology represents a degenerate maltase gene or similar sequences that have no functional role in maltose fermentation. In view of these findings, a recent report by OLIVEIRA *et al.* (1986) is noteworthy. They report the isolation of a DNA fragment from the *MAL4*-constitutive strain 1403-7A capable, in high copy, of complementing a mutation in the *MAL6* activator gene, *mal63*. The restriction map of this fragment shows some similarities to that of the *MAL4* activator gene (*MAL43*) yet the two sequences are distinct both structurally and functionally since the *mal63* transformants carrying this fragment are largely inducible and glucose repression sensitive whereas transformants carrying the *MAL43*-constitutive allele are constitutive and glucose repression insensitive (CHARRON and MICHELS 1987; OLIVEIRA *et al.* 1986). LI (1984) and HALL, YOKOYAMA and CALHOUN (1983) discuss the role of cryptic genes in microbial populations and suggest that these elements add to the "genetic reservoir" of the species because they may become mutationally activated at sometime in the future. Whether or not these other *MAL* related sequences can serve a similar role, or any role, is a question that remains unanswered. We would, however, like to propose an origin for these sequences: interchromosomal exchange of telomeres and telomere-linked sequences. We suggest that an as yet undefined exchange process involving the telomere and telomere-linked sequences of non-homologous chromosomes occurs and that such exchanges lead to the duplication of telomere-linked sequences. In fact, the *X* and *Y'* sequences described by CHAN and TYE (1983) could be the products of just such exchanges. Should such an exchange process occur, the less tightly linked a sequence is to the chromosomal ends the less frequently it would be duplicated. Thus, *MAL* coding sequences as well as noncoding flanking DNA may have become repeated by this proposed telomere exchange process. Once duplicated, the sequences could degenerate as a result of mutagenic processes and this would lead to copies whose function was either lost or altered.

We wish to thank MARIAN CARLSON, AARON MITCHELL, CAROL NEWLON and RICHARD B. NEEDLEMAN for critical reading of this manuscript. We also thank RICHARD NEEDLEMAN for providing yeast strains; MARIAN CARLSON for providing yeast strains and plasmids pFE52, pRB117 and pRB59; BIK-KWOON TYE and CLARENCE CHAN for plasmids YRp120, YRp131A and YRp131B; ED-

WARD FRITSCH for phage EMBL3; STEVE HUGHES for *E. coli* strain K803 and STEVE DELLA PORTA for *E. coli* strains BHB2890 and BHB2888 and for helpful suggestions concerning preparation of packaging extracts. We are also grateful to ROBERT DUBIN for assistance in construction of several *MAL1g* subclones and GENEVIEVE CANNON for tetrad dissections.

This work was supported by U.S. Public Health Service grant GM-28216 from the National Institutes of Health to C.A.M. and by a Board of Higher Education-Professional Staff Congress Research Award.

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Communicating editor: E. W. JONES