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Repeated Family of Genes Controlling Maltose Fermentation in *Saccharomyces carlsbergensis*

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Maltose fermentation in *Saccharomyces* spp. requires the presence of any one of five unlinked genes: *MAL1*, *MAL2*, *MAL3*, *MAL4*, or *MAL6*. Although the genes are functionally equivalent, their natures and relationships to each other are not known. At least three proteins are necessary for maltose fermentation: maltase, maltose permease, and a regulatory protein. The *MAL* genes may code for one or more of these proteins. Recently a DNA fragment containing a maltase structural gene has been cloned from a *MAL6* strain, CB11, to produce plasmid pMAL9-26. We have conducted genetic and physical analyses of strain CB11. The genetic analysis has demonstrated the presence of two cryptic *MAL* genes in CB11, *MAL1g* and *MAL3g* (linked to *MAL1* and to *MAL3*, respectively), in addition to the *MAL6* locus. The physical analysis, which used a subclone of plasmid pMAL9-26 as a probe, detected three *Hin*dIII genomic fragments with homology to the probe. Each fragment was shown to be linked to one of the *MAL* loci genetically demonstrated to be present in CB11. Our results indicate that the cloned maltase structural gene in plasmid pMAL9-26 is linked to *MAL6*. Since the *MAL6* locus has previously been shown to contain a regulatory gene, the *MAL6* locus must be a complex locus containing at least two of the factors needed for maltose fermentation: the structural gene for maltase and the maltase regulatory protein. The absence of other fragments which hybridize to the *MAL6*-derived probe shows that either *MAL2* and *MAL4* are not related to *MAL6*, or the DNA corresponding to these genes is absent from the *MAL6* strain CB11.

Maltose fermentation by the *Saccharomyces* yeasts requires at least two enzymes, maltase (E.C. 3.2.1.20, α,D-glucosidase) and maltose permease. The synthesis of these enzymes is induced by adding maltose to the growth medium. In the absence of inducer, only low basal levels of each enzyme are present. Based on these physiological studies as well as on genetic analyses of the maltose fermentation genes (see below), it has been proposed that a regulatory protein controls the production of both maltase and maltose permease.

The genetic characterization of the genes involved in maltose utilization in the *Saccharomyces* yeast species has identified a family of unlinked loci, *MAL1*, *MAL2*, *MAL3*, *MAL4*, and *MAL6* (14–16). The presence of any one of these loci is sufficient for maltose fermentation. Maltose-nonfermenting mutants which synthesize only basal levels of wild-type maltase have been isolated in a strain carrying the *MAL6* locus, and their mutations have been shown to map to the *MAL6* locus, forming a single complementation group. These noninducible mutants appear to be defective in the regulatory protein controlling the induction of maltase (12). Similar mutants have been isolated in *MAL2* strains, and it has been postulated that all of the *MAL* loci code for at least this regulatory protein (17). No mutations in the maltase structural gene or in the maltose permease gene have yet been identified, despite extensive mutational analysis. One explanation for the inability to isolate structural gene mutations proposes the presence of multiple genomic copies of these genes.

The possibility that the *MAL* loci code for more than just the regulatory protein has been presented (5, 6). Naumov, working with natural isolates of *Saccharomyces* spp., found a complementing gene system involving two complementation groups which he called *MALp* and *MALg*. When two maltose-nonfermenting strains from the two complementation groups were crossed, the diploid was able to ferment maltose. Dissection of such diploids enabled Naumov to identify in his strains two *MAL* genes, of which one was allelic to the *M1* gene described by Winge (and thus allelic to *MAL1*) and the other was allelic to Winge's *M2* gene (and thus allelic to *MAL3*) (6, 13). We refer to

these *MAL* genes as *MAL1g* and *MAL3g*. The *MALp* strains identified by Naumov and used to complement these *MALg* strains were shown to carry a *MALp* gene which also mapped to the *MAL1* locus and was therefore designated as *MAL1p*. Genetic analysis of a maltose regulatory mutant (*mal6-17*) isolated by ten Berge showed it to be complemented by *MAL1p* but not by *MAL1g*, implying that *MAL1p* codes for a maltose regulatory protein which is functionally equivalent to the regulatory protein coded for by the *MAL6* locus (7).

Recently, a segment of DNA from a *MAL6* strain (CB11) has been cloned into a yeast plasmid vector to form plasmid pMAL9-26 (3). This plasmid is able to transform a maltose-nonfermenting strain into a maltose fermentor. It was also demonstrated that this segment contains a structural gene for maltase. In the absence of any structural gene mutations, and in light of the proposed multiple copies of the structural gene, the possibility that this fragment may in fact be unlinked to *MAL6* must be considered. Rigorous proof that any cloned DNA fragment actually represents the *MAL6* locus requires that it be mapped to that locus. The observed complementation by pMAL9-26 may be due to a variety of causes, including a gene dosage effect resulting from the use of a multicopy plasmid.

We show here that the cloned segment in the plasmid pMAL9-26 does indeed represent *MAL6*. *MAL6* is therefore a complex locus consisting of at least two genes, the regulatory gene and the maltase structural gene. We demonstrate that the 7.3-kilobase (kb) *Hin*dIII fragment cloned from the *MAL6* region contains *MALg* activity as well as the maltase structural gene as shown by Federoff et al. (3). Whereas this finding suggests that the *MALg* function is dependent upon the presence of a structural gene, it is not yet possible to identify the *MALg* function as coding for the maltase structural gene.

Given the functional identity of *MAL1* through *MAL4* and *MAL6* and the availability of a *MAL6* probe, it was also of interest to explore the possibility that these genes are related at the nucleotide level. We demonstrate that strain CB11 contains multiple unlinked DNA segments with significant homology to *MAL6*. Two of these fragments segregate with active *MALg* genes identified in this strain by our genetic analysis. These active *MALg* genes are linked to the *MAL1* and *MAL3* loci and therefore represent *MAL1g* and *MAL3g* genes. At least three of the genes of the *MAL* family, *MAL1*, *MAL3*, and *MAL6*, are therefore related at the nucleotide level. No indication of the presence of *MAL* sequences homologous to the *MAL6* probe was found at other positions in the genome. The *MAL2* and *MAL4* loci either lack homology with *MAL6* or are deleted for the segment corresponding to the probe.

MATERIALS AND METHODS

Strains. The *MAL1p*, *MAL1g*, and *mal*^{0} tester strains were constructed from homothallic strains kindly provided by G. Naumov, Institute for the Genetics of Microorganisms, Moscow. The *MAL6* strain, CB11 (**a** *MAL6 ade1*), was obtained from the collection of A. M. A. ten Berge and is identical to that used by Federoff et al. (3). However, although our CB11 strain was derived from the same diploid (NCYC74), it is not identical to the CB11 (α*MAL6 lys2*) strain characterized by Naumov (7). A *MAL1* standard strain, 4059, was provided by R. Haekel. The standard *MAL2* (1453-3B), *MAL3* (1412-4D), and *MAL4* (1403-7A) strains were obtained from the Berkeley Yeast Stock Center collection, Berkeley, Calif. A large number of tester strains were constructed in the course of this study. Key strains will be provided to the Berkeley Yeast Stock Center. Plasmid YIP5 was kindly supplied by Tom Donahue.

Genetic analysis. Standard techniques of mating, sporulation, dissection, and tetrad analysis were used (4). Maltose fermentation was determined in 5-ml Durham tubes of 1% yeast extract–1% peptone–1% maltose. Fermentation was indicated by the production of gas 1 to 3 days after inoculation.

DNA preparation. Two procedures were used for preparing total genomic DNA from *S. carlsbergensis*. One was a modification of the method of Cryer et al. (1), and the other, for preparations from small culture volumes, was described by Sherman et al. (9).

Restriction digestion and Southern analysis. Restriction digestion was done under conditions recommended by the commercial supplier (Bethesda Research Laboratories, Bethesda, Md.). Fragments were separated on 0.8% horizontal agarose gels. The gels were treated as described by Southern (10), and the nitrocellulose filters were hybridized to ^{32}P-labeled probes and exposed to Kodak XAR film at −70°C with an intensifying screen.

Nick translation. The probe DNA was labeled by nick translation with [^{32}P]dCTP (Amersham Corp., Arlington Heights, Ill.) according to procedures described by Rigby et al. (8).

RESULTS

Our goal was to define genetically and physically the *MAL* genes present in the *MAL6* strain CB11. This strain was used as the DNA source in the construction of the clone pMAL9-26, which contains the maltase structural gene. A *MAL6* strain related to CB11 was analyzed genetically by Naumov and found to contain multiple *MALg* genes unlinked to *MAL6* (7). Therefore, as a first step toward the complete analysis of CB11, we decided to determine whether any unlinked *MALg* genes were present in CB11. Before proceeding with the complete genetic analysis of CB11 we wish to introduce a new notation which we have found extremely

useful for concisely describing the genotype of *MAL* strains.

Genetic notation for the *MAL* loci. The large number of loci involved in maltose metabolism along with the presence of unlinked *MALg* genes greatly complicates the notation of genotypes. As will be made clear by the results presented below, the standard *MAL* strains available through the Yeast Genetics Stock Center, or described in the literature as *MAL2* or *MAL6*, etc., are only partially defined genetically with regard to the *MAL* loci. Complete genotypic analysis of these strains requires the use of the *MAL1p* and *MAL1g* strains, which have only recently become available. Using such strains, we demonstrated that the standard maltose-fermenting *MAL* strains contain, in addition to the designated dominant *MAL* locus, additional unlinked *MALg* genes. This is shown here (i.e., in this paper) for a *MAL6* strain and also holds true for other *MAL* strains obtained from the Yeast Genetics Stock Center (C. Michels and R. B. Needleman, manuscript in preparation). The following notation has been established.

(i) The standard allele tester strains for determining the presence of *MALg* or *MALp* function are the *MAL1p* and *MAL1g* alleles obtained from G. Naumov. Strains complementing the *MAL1g* strain are said to have *MALp* function; strains complementing the *MAL1p* strain are said to have a *MALg* function.

(ii) Only active genes are noted. A strain written as *MAL1p* indicates that no additional *MALp* or *MALg* genes are present at any locus.

(iii) Strains which are maltose negative and which lack genes capable of complementing either *MAL1p* or *MAL1g* are written as mal^0. This convention differs from previous notations which have used mal^0 to denote maltose segregational nonfermenters. As shown elsewhere (Michels and Needleman, in preparation), such segregational nonfermenters often contain *MALg* genes.

(iv) When the genotype of a maltose-fermenting strain is written, e.g., as *MAL6* (mal^0), the parentheses around (mal^0) are used to indicate that there are no genes unlinked to *MAL6* that can complement either *MAL1p* or *MAL1g*. Such strains were constructed by repeatedly backcrossing the *MAL* strains obtained from the Yeast Genetics Stock Center to mal^0 strains until no segregating *MALg* loci could be detected.

Genetic analysis of a standard *MAL6* strain, CB11. Strain CB11, a standard *MAL6* strain, was crossed to strain 6-2A, a maltose-nonfermenting mal^0 strain. In nine tetrads resulting from this cross (cross W208), maltose fermentation segregated 2:2. When the two negative segregants in each tetrad were crossed to a *MAL1p* strain, 13 segregants complemented the *MAL1p* strain to give maltose fermentation, indicating the presence of *MALg* genes unlinked to *MAL6*. The segregation of *MALg* in these nonfermenters gave five tetrads with 2 *MALg*:0 mal^0, three tetrads with 1 *MALg*:1 mal^0 and one tetrad with 0 *MALg*:0 mal^0, suggesting that more than one *MALg* gene was involved. This result was confirmed by detailed genetic analysis as presented below.

Segregant W208-2B of diploid W208. Strain W208-2B, a nonfermenter, was crossed to the mal^0 strain W106-1B (diploid W243). In six complete tetrads, *MALg* segregated 2:2, indicating the presence of one *MALg* gene. When the *MALg* segregant from cross W243 (W243-2B) was crossed to a *MAL*1 (mal^0) strain, W203-3B, all 31 maltose-nonfermenting segregants resulting from this cross complemented *MAL1p*. Thus the *MALg* gene present in W208-2B is linked to *MAL*1 and is therefore *MAL1g*.

Segregant W208-2C of diploid W208. Strain W208-2C, a maltose fermenter, was crossed to mal^0 strain W240-1C. Of 14 maltose-nonfermenting segregants, all were mal^0. W208-2C therefore lacks unlinked *MALg* genes and is therefore *MAL6* (mal^0).

Segregant W208-2A of diploid W208. Strain W208-2A, a maltose nonfermenter, was crossed to the mal^0 strain 3-2B (W242). When dissected, it yielded eight asci which showed a 4 *MALg*:0 mal^0 or a 3 *MALg*:1 mal^0 segregation pattern, clearly indicating the presence of more than one *MALg* gene. Tetrad 3 of diploid W242, which gave four *MALg* segregants, was subjected to further analysis by mating each segregant to a mal^0 strain. The results (detailed below) indicated that each segregant of tetrad 3 contained only one *MALg* gene. When W242-3B was crossed to a *MAL1* (mal^0) strain, W203-7B, 23 of the 23 maltose-nonfermenting spores were *MALg*. Thus W242-3B has the *MAL1g* gene. A strain containing the *MALg* gene derived from W242-3D was crossed with a *MAL3* (mal^0) strain, W48-2C, and four-spored and three-spored tetrads were analyzed. Maltose fermentation segregated 2:2 (in four-spored tetrads), and all 38 maltose-nonfermenting segregants were *MALg*. W242-3D, thus, carries the *MAL3g* gene. The genotype of W208-2A is, therefore, *MAL1g MAL3g*.

In summary the genotypes are: 208-2A, *MAL1g MAL3g*; 208-2B, *MAL1g*; and 208-2C, *MAL6* (mal^0). CB11 therefore has the genotype *MAL6 MAL1g MAL3g*.

Southern analysis of strain CB11. Figure 1 shows the restriction map of the major *Hin*dIII fragment of the pMAL9-26 *S. carlsbergensis* DNA insert. This map differs slightly from that previously published (3) in the location of a small 350-base pair *Eco*RI fragment. The

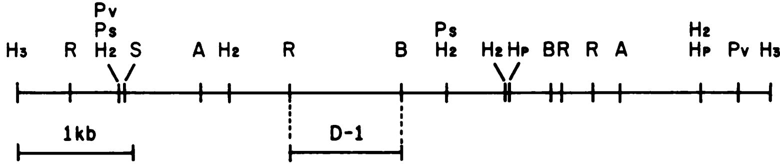

FIG. 1. Restriction endonuclease map of the major *Hin*dIII fragment from plasmid pMAL9-26. The D1 probe was constructed by subcloning the *Eco*RI fragment containing D1 into pBR325 and then deleting the region from the *Bgl*II side of the insert to the *Bam*HI side of the vector. A, *Ava*I; B, *Bgl*II; H_2; *Hin*cII; H_3, *Hin*dIII; Hp, *Hpa*I; Ps, *Pst*I; Pv, *Pvu*II; R, *Eco*RI; S, *Sal*I.

*Hin*dIII fragment was subcloned into the nonreplicating vector YIP5 (5) to form plasmid Y6. Plasmid Y6 behaves as a replicating plasmid in *S. carlsbergensis* presumably owing to the presence of a chromosomal replication origin on the *Hin*dIII fragment. When introduced into either a *mal*^{0} or a *MALg* strain, the strain remained unable to ferment maltose. However, when transformed into a *MAL1p* strain, the plasmid complemented to give maltose fermentation, indicating that *MALg*-complementing activity was present on the cloned *Hin*dIII fragment.

Since this cloned fragment has *MALg* activity and multiple *MALg* genes are present in CB11, it was of interest to determine whether the fragment cloned was unique in the genome and, if not, whether it could be used as a probe to distinguish physically the multiple *MALg* genes present in CB11 by using Southern analysis. Total DNA was prepared from CB11, from 6-2A (the *mal*^{0} parent), and from four tetrads resulting from the diploid W208. Southern analysis of these strains after digestion with *Hin*dIII is shown in Fig. 2. The probe used, D1, is an internal *Eco*RI-*Bgl*II subclone of the 7.3-kb *Hin*dIII fragment and is shown in Fig. 1.

Initial observation of the results showed that CB11 had at least two *Hin*dIII genomic fragments with significant homology to the probe and that the *mal*^{0} parent did have homology to the probe despite its lack of all *MALp*- and *MALg*-complementing activity. No common bands were present in both parents. CB11 had a band at approximately 10.7 kb (the A band) and a very intense band at 7.3 kb (the B band). Strain 6-2A had one band at 7.0 kb (the C band). Given the existence of these restriction polymorphisms, it becomes possible to correlate maltose fermentation and function with the presence of particular *Hin*dIII fragments. Bands A and C can be seen to segregate 2:2 in the selected tetrads shown in Fig. 2. Band B represents a doublet, which we will refer to as bands B and B′.

Figure 2 also indicates which segregants were maltose fermenters, and therefore contained the *MAL6* locus. Based on these four tetrads and three others (data not shown) we can correlate maltose fermentation with the presence of one of the 7.3-kb *Hin*dIII bands (B or B′). Segregants containing only band C, e.g., W208-1A and W208-4D, are *mal*^{0} like their 6-2A parent. Figure

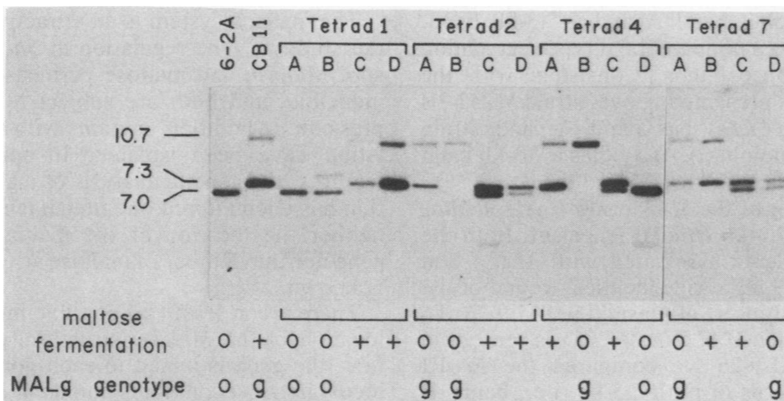

FIG. 2. Southern analysis of segregants from the diploid W208 (CB11 × 6-2A). CB11 is a standard *MAL6* strain. Strain 6-2A is a *mal*^{0} strain (see text). Total DNA preparation from the two parent strains and 16 segregants were digested with *Hin*dIII. The fragments were separated on an 0.8% agarose gel and analyzed by the method of Southern (10). The nitrocellulose filters were probed by using a plasmid containing region D1 (see Fig. 1).

2 also shows the results of *MAL1p* complementation tests done for each maltose-nonfermenting segregant, indicating the presence or absence of *MALg* genes. All maltose-nonfermenting segregants that contained *MALg* genes also contained a 7.3-kb band (B or B′), band A, or both. No segregant entirely lacked homology to the probe. This can be explained by the observation that bands A and C segregated in repulsion. Therefore they are alleles or closely linked. The segregation patterns of A and the B-B′ doublet in the seven tetrads analyzed provides no indication of linkage. A possible hypothesis, which we prove below, is that band B represents the *MAL6* locus, and that bands A and B′ represent the unlinked *MALg* genes.

The genotype of tetrad 2 (Fig. 2) was detailed above. Segregant W208-2B, shown to carry only the *MAL1g* locus, can be seen in Fig. 2 to contain only band A. Southern analysis of segregants from the diploid W243 (W208-2B × mal^0) shows that band A segregated with the *MAL1g* gene in all six tetrads tested (data not shown). Thus, band A is linked to *MAL1g*.

Segregant W208-2C carries only the *MAL6* locus and lacks all unlinked *MALg* genes. From Fig. 2, W208-2C is seen to contain a 7.3-kb fragment and the 7.0-kb fragment seen in all mal^0 strains. Thus, one of the 7.3-kb fragments is associated with the *MAL6* locus.

Segregant W208-2A was genetically shown to carry both the *MAL1g* and *MAL3g* loci. In Fig. 2, this segregant can be seen to contain at least two *Hin*dIII fragments, band A and a 7.3-kb band, homologous to the D1 probe. Segregant W208-2A was mated to a mal^0 strain to give diploid W242. Southern analysis of the segregants from tetrads 3, 5, and 7 of diploid W242 is shown in Fig. 3. Strain W242-2A can be seen to contain only two *Hin*dIII fragments homologous to the D1 probe: band A and a 7.3-kb band. Tetrad 3 shows a nonparental ditype segregation pattern for these bands. Consistent with the genetic results presented above, strain W242-3B (containing *MAL1g*) has band A, and strain W242-3D (containing *MAL3g*) has a 7.3-kb band and band C (the mal^0-associated band).

Identification of the *MAL* genes corresponding to the cloned 7.3-kb *Hin*dIII fragment. Both the *Hin*dIII fragments associated with *MAL6* and *MAL3g* are 7.3 kb, a size identical to that of the cloned *Hin*dIII insert of plasmid pMAL9-26. To identify whether *MAL6* or *MAL3g* is present in plasmid pMAL9-26 we compared the *Eco*RI digestion patterns of both 7.3-kb (i.e., bands B and B′) genomic fragments with that of plasmid pMAL9-26 (Fig. 4). Tetrad 7 of diploid W208 (Fig. 2) shows the two 7.3-kb fragments in a nonparental ditype segregation pattern. Segregant 7B ferments maltose and thus carries the

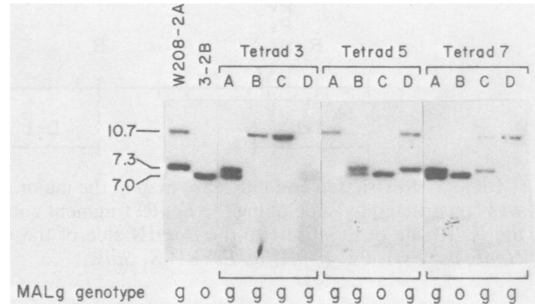

FIG. 3. Southern analysis of segregants from the diploid W242 (W208-2A × 3-2B). W208-2A is a maltase-nonfermenting segregant of diploid W208 (see Fig. 2). Strain 3-2B is mal^0. Total DNA preparations from the parent strains and segregants from three representative tetrads were digested with *Hin*dIII and subjected to Southern analysis, as described for Fig. 2. The filters were probed with the D1-containing plasmid.

MAL6 locus; segregant 7A is a nonfermenter and carries the *MAL3g* gene. Both contain the *MAL1g* band A. A strain containing the same fragment as plasmid pMAL9-26 should after digestion release a 2.55-kb fragment spanning the D1 region used as the probe in these experiments. *Eco*RI-*Hin*dIII digests of strains W208-7A and W208-7B, probed with D1, gave two regions of homology. The upper fragment, also found in W208-4B, clearly resulted from band A. This lower band differed in these two strains, and only W208-7B could be seen to release the expected 2.55-kb fragment D. Therefore, the genomic fragment cloned in plasmid pMAL9-26 is, in fact, the *MAL6* locus.

DISCUSSION

The maltase system is an attractive model for the study of gene regulation in *Saccharomyces* spp. Maltase and maltose permease are highly inducible, and both are subject to glucose repression. In addition, mutants with altered regulation have been isolated. In spite of these features, the genetic analysis of maltose regulation has encountered one fundamental obstacle: neither the location of the maltase structural gene nor the number of maltase structural genes is known.

There are at least two possible models for the location of the maltase structural gene. In the first, the gene is linked to each dominant *MAL* locus; in the second, it is unlinked. Both models are consistent with the genetic data. Maltose fermentation, while requiring the presence of at least three genes (the regulatory gene, the maltose structural gene, and the gene for maltose permease), segregates as a single gene in crosses

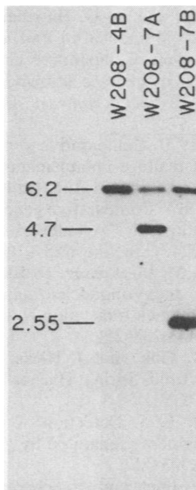

FIG. 4. Comparison of the *Eco*RI digestion pattern of the two 7.3-kb *Hin*dIII fragments present in CB11 (the *MAL6* parent strain). DNA preparations from segregants W208-4B, W208-7A, and W208-7B (see Fig. 2) were digested with *Hin*dIII and *Eco*RI and subjected to Southern analysis as described for Fig. 2). D1 was used as the probe.

with nonfermenting strains. This implies that either a maltase structural gene is linked to each dominant *MAL* locus or that an active structural gene is common to both the maltose-fermenting and the maltose-nonfermenting parents. This latter explanation is more than just a theoretical possibility; all maltose nonfermenters of the *MALg* genotype examined have basal levels of authentic maltase and must therefore have an active maltase structural gene (Needleman and Michels, unpublished data).

The possibility that more than one maltase structural gene is present in a given *MAL* strain is suggested by two lines of evidence. The first is that no mutants having mutations in the maltase structural gene have been isolated despite extensive searches. The second is derived from the following experiment. We crossed a single maltose-negative strain to both a *MAL6* and a *MAL2* strain. Eight maltose-negative spores from each cross were tested for maltase activity; all had basal levels of authentic maltase. This suggests that the maltase structural gene present in both the *MAL6* and the *MAL2* strain is homozygous to the structural gene present in the maltose-nonfermenting strain. Clearly, either there are two structural genes in the maltose nonfermenter allelic to both *MAL2* and *MAL6*, or else both the *MAL2* and the *MAL6* strains have a single maltase structural gene which is unlinked to either *MAL6* or *MAL2* and is identical to the structural gene present in the nonfermenting strain.

If we wish to account for the inability to isolate mutants in the maltase structural gene by postulating that in a *MAL6* strain (like CB11) there are, in addition to the *MAL6*-linked maltose structural gene, other unlinked maltase structural genes, then certain constraints are placed on the nature of these genes. These genes must be under the control of the *MAL6* regulatory protein and must be quite similar in sequence, since several physical studies have failed, with one exception, to distinguish between the enzymes produced in strains carrying a single *MAL* gene (2). To investigate the location and number of the maltase structural genes, we have conducted genetic and physical analyses of strain CB11, a strain used extensively in the analysis of maltose regulation (A. M. A. ten Berge, Ph.D. Thesis, Rijkuniversiteit te Utrecht, Utrecht, The Netherlands, 1974). The genetic analysis utilizes *MALp* and *MALg* tester strains recently made available by G. Naumov; the physical analysis relies on the recent isolation of a maltase structural gene from CB11 (3).

The genetic analysis has established the complete genotype of CB11. CB11 has three genes involved in maltose metabolism, namely, *MAL6*, *MAL1g*, and *MAL3g*. Strains containing the *MAL6* gene alone are able to ferment maltose, whereas those carrying the *MAL1g* or *MAL3g* genes alone are unable to ferment maltose unless they are supplied with a complementing *MALp* gene. In the absence of a complementing *MALp* gene, such *MALg* genes are cryptic.

The physical analysis used a DNA fragment of CB11 that had been cloned onto a replicating *S. carlsbergensis* plasmid. This fragment had been shown to contain the maltose structural gene (3). The physical analysis had demonstrated the existence of three genetically unlinked genomic *Hin*dIII fragments from CB11 that show significant homology to the *S. carlsbergensis* DNA contained on plasmid pMAL9-26 and homology in particular to a subclone, D1. Each of these fragments segregates with one of the *MAL* genes defined above: a 10.7-kb fragment with *MAL1g*; a 7.3-kb fragment with *MAL3g*; and a 7.3-kb fragment with *MAL6*. Recently we have shown that the *Eco*RI fragment from which probe D1 was derived hybridizes to two maltose-inducible mRNAs. D1 therefore contains sequences linked to the maltase structural gene.

Since the D1 probe hybridized to three distinct, unlinked fragments of CB11 DNA, the possibility remained that the clone pMAL9-26 contained DNA from a structural gene unlinked to *MAL6*. The observed transformation of a maltose-negative strain into a maltose-positive strain is by itself inadequate evidence for the cloning of *MAL6*. To resolve this difficulty, Southern analysis of *Eco*RI digests of strains

containing *MAL3g* and *MAL6* were compared; these demonstrated that the cloned fragment in plasmid pMAL9-26 is in fact the *MAL6* locus.

These results lead us to propose the following model for the organization of the *MAL* genes. Each *MAL* locus is a gene complex consisting of at least two genes, a maltase regulatory gene and a maltase structural gene. Preliminary evidence suggests that another gene product under maltose control (presumably the permease) is also present. We postulate that strains carrying a single *MAL* gene frequently have, in addition to the structural gene linked to the dominant *MAL* gene, additional cryptic *MAL* genes. These cryptic *MAL* genes (e.g., *MALg*) lack a regulatory gene but have structural genes which are highly homologous to those present at the dominant *MAL* locus, and these genes can be turned on by the regulatory protein linked to the dominant *MAL* allele.

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LITERATURE CITED

1. **Creyer, D. R., R. Eccleshall, and J. Marmur.** 1975. Isolation of yeast DNA. Methods Cell Biol. 7:39–44.
2. **Eaton, N. R., and F. K. Zimmermann.** 1976. Thermal inactivation of maltase and its application to temperature-sensitive mutants of yeast. Mol. Gen. Genet. **148:**199–204.
3. **Federoff, H., J. D. Cohen, T. R. Eccleshall, R. B. Needleman, B. A. Buchferer, J. Giacalone, and J. Marmur.** 1982. Isolation of a maltose structural gene from *Saccharomyces carlsbergensis*. J. Bacteriol. **149:**1064–1070.
4. **Mortimer, R. K., and D. C. Hawthorne.** 1975. Genetic mapping in yeast. Methods Cell Biol. **6:**221–233.
5. **Naumov, G. I.** 1969. Comparative genetics of yeast. I. Complementation of maltose utilizing genes in maltose-negative species of *Saccharomyces*. Genetika **5:**142–149. (In Russian)
6. **Naumov, G. I.** 1970. Comparative genetics of yeast. IV. Identification of maltose complementing factors in *Saccharomyces*. Genetika **6:**121–124. (In Russian)
7. **Naumov, G. I.** 1976. Comparative genetics of yeast. XVI. Maltose fermenting genes in *Saccharomyces carlsbergensis* strain NCYC74. Genetika **12:**87–100. (In Russian)
8. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113:**237–251.
9. **Sherman, F., G. Fink, and J. Hicks.** 1981. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
11. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. U.S.A. **76:**1035–1039.
12. **ten Berge, A. M. A., G. Zoutewelle, and K. W. van de Poll.** 1973. Regulation of maltose fermentation in *Saccharomyces carlsbergensis*. I. The function of the gene *MAL6* as recognized by *mal6* mutants. Mol. Gen. Genet. **123:**233–246.
13. **Winge, O., and C. Roberts.** 1948. Inheritance of enzymatic characters in yeasts, and the phenomenon of long-term adaptation. C. R. Trav. Lab. Carlsberg **24:**263–315.
14. **Winge, O., and C. Roberts.** 1950. The polymeric genes for maltose fermentation in yeasts and their mutability. C. R. Trav. Lab. Carlsberg **25:**36–83.
15. **Winge, O., and C. Roberts.** 1950. Identification of the gene for maltose fermentation in *Saccharomyces italicus*. Nature (London) **166:**1114.
16. **Winge, O., and C. Roberts.** 1955. Identification of the maltase genes in some American haploid and European diploid yeasts. C. R. Trav. Lab. Carlsberg **25:**331–340.
17. **Zimmerman, F. K., and N. R. Eaton.** 1974. Genetics of induction and catabolite repression of maltase synthesis in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **134:**261–272.