THE EFFECTS OF THREE DIFFERENT *MAL* LOCI ON THE REGULATION OF MALTASE SYNTHESIS IN YEAST

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ABSTRACT

Inbred haploid strains of *Saccharomyces cerevisiae* carrying *MAL1*, *MAL2* or *MAL6* in a common background have been crossed to each other and to strains carrying no active *MAL* loci. The kinetics of maltase induction and the induced maltase levels have been examined in the inbred strains and in haploid segregants of the crosses. Differences have been found in the kinetics of induction and induced maltase levels that segregate with the different *MAL* loci. In the strains tested, the relative rates of maltase induction were *MAL2*> *MAL6*>>*MAL1*; the relative induced maltase levels were *MAL2*>*MAL6*~ *MAL1*. These results indicate that *MAL1*, *MAL2* and *MAL6* are (or include) regulatory genes that control the accumulation of the enzymes of maltose fermentation.

THERE are at least five unlinked genes that control the ability of yeast to produce maltase (and maltase permease) in response to maltose: *MAL1*, *MAL2*, *MAL3*, *MAL4* and *MAL6* (reviewed by BARNETT 1976). Strains carrying an active allele of any one of these loci are inducible; strains carrying inactive alleles at all loci are uninducible, but produce basal levels of maltase (KHAN, ZIMMERMAN and EATON 1973; TEN BERGE, ZOUTEWELLE and VAN DE POLL 1973).

The role of the *MAL* genes has been investigated genetically in two ways, either by analyzing mutations at a single MAL locus (KHAN, ZIMMERMAN and EATON 1973; TEN BERGE, ZOUTEWELLE and VAN DE POLL 1973; TEN BERGE *et al.* 1973; ZIMMERMAN and EATON 1974), or by comparing the effects of different MAL loci (RUDERT and HALVORSEN 1962; TAURO and HALVORSON 1966). As an example of the first approach, TEN BERGE and his associates have analyzed the effects of mutations in the *MAL6* locus. They have found that both noninducible and constitutive mutations map in the *MAL6* locus (TEN BERGE, ZOUTEWELLE, VAN DE POLL 1973; TEN BERGE *et al.* 1973) and that two strains that are temperature sensitive for maltase induction contain maltase indistinguishable from that of the wild type (TEN BERGE, ZOUTEWELLE and VAN DE POLL 1973).

These and similar results with other *MAL* loci (KHAN, ZIMMERMAN and EATON 1973; ZIMMERMAN and EATON 1974) are consistent with the proposal that the *MAL* genes are regulatory loci that code for a protein required for maltase induction (TEN BERGE, ZOUTEWELLE and VAN DE POLL 1973; ZIMMERMAN and EATON 1974). These results do not rule out the possibility that the *MAL* genes are

complex loci that also include the structural gene(s) for the enzymes of maltose metabolism, as proposed by others (OSHIMA 1967; NAUMOV 1971). However, no mutations affecting the structure of maltase or maltose permease have as yet been mapped to the *MAL* loci.

As an example of the second approach, HALVORSON's laboratory has compared the effects of several different *MAL* loci on the synthesis of maltase. They have reported that strains carrying different active *MAL* loci produce different amounts of maltase (RUDERT and HALVORSON 1962) and that they do so at different times in the cell cycle (TAURO and HALVORSON 1966). On the basis of their results they have proposed that the *MAL* loci are structural genes for maltase, but their data are also consistent with a regulatory role for the *MAL* loci.

From all the genetic evidence available, it seems likely that the different *MAL* loci represent a series of wild-type regulatory loci that may control not only the level of maltase production, but the timing of maltase synthesis during the cell cycle (TAURO and HALVORSON 1966). Since mutants affecting the level and timing of maltase production would be extremely difficult to obtain directly, we have chosen to reinvestigate the existing strains carrying different *MAL* loci. In this paper we describe experiments designed to determine (1) the extent of the differences in maltase production between the strains, and (2) whether the differences can be attributed to the *MAL* genes themselves. For these purposes, a series of inbred strains has been constructed so that each strain carries a single active *MAL* locus (*MAL1*, *MAL2* or *MAL6*) in a common background. The regulation of maltase synthesis has been examined in these strains and in segregants from crosses between them. Our results confirm previous findings that strains carrying different *MAL* loci produce different levels of maltase (RUDERT and HALVORSON 1962) and demonstrate that the *MAL* loci themselves are responsible for these differences, as well as for differences in the kinetics of maltase induction.

MATERIALS AND METHODS

Strain construction and genetic analysis: Haploid strains carrying *MAL1*, *MAL2* or *MAL6* were crossed three times to a small number of closely related standard haploid Mal$^-$ strains, as previously described (MOWSHOWITZ 1979). The segregants resulting from these crosses used in this work are listed in Table 1. All segregants used were of the genotype *mgl1 mgl2 mgla* and of phenotype MglB+ MglC+. The exact number of active *MGLB* and *MGLC* loci in each strain is not known. Care was taken to standardize the background with respect to the *MGL* genes because it has been reported that these genes, which control induction of α-methyl glucosidase, may also affect regulation of maltase (BARNETT 1976). The common MGL background used, *mgl1 mgl2 mgla*, MgB+ MglC+, was chosen largely because it most closely resembles that used in previous experiments on the differences between strains carrying different *MAL* loci (MOWSHOWITZ 1979; RUDERT and HALVORSON 1962).

Inbred haploid strains carrying *MAL1*, *MAL2* or *MAL6* were crossed to each other to produce diploids heterozygous for two different *MAL* loci, as listed in Table 2. The resulting diploids were then sporulated and subjected to tetrad analysis, so that segregation of the *MAL* loci could be correlated with any difference in maltase production. Tetrads obtained from these crosses that contained four maltase fermenting spores (4:0 tetrads) were analyzed further to identify the *MAL* locus carried by each segregant, as follows: each segregant was backcrossed to one or more

TABLE 1

α-Glucosidase levels in inbred haploid strains carrying MAL1, MAL2 *or* MAL6

Rounds of inbreeding*	*MAL1* Strain	Δmaltase† / Δgrowth	*MAL2* Strain	Δmaltase / Δgrowth	*MAL6* Strain	Δmaltase / Δgrowth
1	M37–1–2A	NT	M30–11C	5.8	M38–III–5C	2.4
			M30–11B	NT		
2	M88–10A	2.5	M53–13B	5.4	M85–7D	2.7
3	M93–11B	2.0	M59–5A	6.3	M103–9B	3.3
	11C	2.0	5B	5.8	9C	3.3
	4C	3.1	6A	6.5	3B	3.3
	4D	1.8	6C	6.5	3D	3.0
	9C	NT				
	19B	NT				
Average		2.28		6.05		3.05

* The strain listed in round 1 or 2 was used as the Mal+ parent for the succeeding round of inbreeding. M30–11B was used as the Mal+ parent for round 2 of inbreeding for the *MAL2* strains.

† $10 \times \Delta$ units of maltase/ml/Δ Klett units; obtained from slopes of differential growth curves similar to those in Figure 1. All cultures grown in broth by Method A, as described in MATERIALS AND METHODS.

tester strains carrying a single known *MAL* locus and the haploid progeny were examined by random spore analysis. The *MAL* locus carried by each segregant was deduced from the proportion of Mal- progeny observed (see RESULTS and Table 4).

The standard genetic methods and media described by SHERMAN, FINK and LAWRENCE (1972) were used for mating, sporulation and tetrad analysis, except that maltose and α-methy glucoside fermentation were scored on indicator plates (1% yeast extract, 2% peptone, 2% sugar, 0.033% bromocresol purple indicator, 1.5% agar) supplemented with 10 μg of ethidium bromide/ml to prevent residual growth of nonfermenting strains. Since some Mal+ strains (primarily *MAL1*) did not grow well on indicator plates, some poorly growing strains were mated with standard Mal- strains and the diploids scored on indicator plates. *MGL* genotypes and/or phenotypes were determined by complementation tests, as previously described (MOWSHOWITZ 1979).

Random-spore analysis was carried out as follows: A culture of each diploid was sporulated, digested with glusulase to release the spores, diluted (but not sonically treated) and spread on plates containing prespore medium by standard methods described in SHERMAN, FINK and LAWRENCE (1972). Prespore medium was used because it contains high levels of glucose, which promote red pigment accumulation in *ade1* mutants. All diploids to be subjected to random spore analysis were constructed so that *ade1* and at least one other auxotrophic marker unlinked to the *MAL* loci were segregating in the cross. Presumptive haploid segregants were selected on the basis of their small colony size and/or red color. The presumed haploids were then scored for ability to grow on minimal medium and for the ability to ferment maltose. Only strains unable to grow on minimal medium were counted as segregants.

TABLE 2

Diploid Saccharyomyces strains used

Diploid	*MAL1* parent	*MAL2* parent	*MAL6* parent
M118	—	M59–5A	M103–9C
M119	M93–9C	M59–5A	—
M120	M93–19B	—	M103–9C

Media and growth conditions: Minimal medium contained 0.6% nitrogen base without amino acids (Difco) plus a carbon source as indicated. It was supplemented with 40 mg each of adenine sulfate, tryptophan and histidine hydrochloride, with 60 mg lysine hydrochloride and 60 to 120 mg leucine per liter.

Maltose broth consisted of the semidefined medium of VAN WIJK described in TEN BERGE, ZOUTEWELLE and VAN DE POLL (1973): 2 g of KH_2PO_4, 6 g of $(NH_4)_2SO_4$, 2.5 g of yeast extract and 0.25 g of $MgSO_4 \cdot 7H_2O$ per liter of medium, containing 2% maltose and supplemented with 20 mg each of adenine sulfate, tryptophan, and histidine hydrochloride and 30 mg each of leucine and lysine hydrochloride per liter.

Cell cultures were grown on a roller at 30°, and cell growth was monitored with a Klett-Summerson colorimeter equipped with a red filter. In the range used, one Klett unit corresponds to approximately 2×10^5 cells/ml.

For differential growth curves, cells were grown overnight to log phase and either followed without further manipulations (method A) or diluted and grown for two generations before use (method B). Results with both methods were indistinguishable.

For induction curves, cells were pregrown in minimal medium containing 3% glycerol and 2% ethanol to late log or early stationary phase; then they were diluted in the same medium and grown overnight to log phase. Maltose was added to 1% to begin induction. For measurements of the speed of induction, cells were pregrown in minimal medium containing glycerol and ethanol and either induced as above or diluted and grown for 2 to 5 hours in the same medium before induction. Results with both methods were indistinguishable.

Maltase assays: Cell suspensions were spotted onto filter-paper disks, dried and assayed for α-glucosidase activity by hydrolysis of p-nitrophenyl-α-D-glucoside (PNPG), as previously described by MOWSHOWITZ (1976). For quantitative assays, individual filters were incubated from 1 to 90 min in scintillation vials containing a buffered solution of PNPG (0.05 M sodium phosphate buffer, pH 6.9, containing 1 mg/ml PNPG), and the amount of nitrophenol released from PNPG was measured by the absorbance at 410 nm. The activity is expressed in the following arbitrary units: 1 U corresponds to 0.0667 absorbance per min at 410 nm. The value previously reported by MOWSHOWITZ (1979) of 4 optical density units per min was in error. Maltase levels measured in this way were generally reproducible from experiment to experiment, but some variation was found, especially when the experiments were repeated at widely spaced intervals. It has since been found that the variation can be reduced, though not totally eliminated, by inclusion of 1 mM EDTA in the assay buffer.

For measurements of the speed of induction, cell suspensions were assayed qualitatively for maltase activity after 4 to 5 hours of induction. 10 μl samples of each induced culture were spotted in a pattern onto Whatman No. 1 filter paper and dried overnight at room temperature. The filter paper was then wetted with 0.05 M sodium phosphate buffer, pH 6.9, containing 1 mg/ml PNPG, placed in a closed petri dish or wrapped in Saran wrap, and incubated at room temperature for up to 2 hours. Maltase activity in each culture was measured by the speed of appearance of yellow color in the corresponding spot. Standard laboratory strains carrying *MAL1*, *MAL2* and *MAL6* were clearly distinguishable by this procedure; after 4 hours of induction, cultures of *MAL2* strains turned yellow in 2 to 5 min, *MAL6* strains turned yellow in 10 to 30 min, and *MAL1* strains gave no detectable color in 90 min.

α-Glucosidase activity measured by hydrolysis of PNPG has been equated with maltase on the basis of previous findings that most of the α-glucosidase in maltose-grown strains with our standard MGL genotype is maltase (GORMAN 1963; TEN BERGE 1973) and on direct measurements on extracts of our standard strains (results not shown).

RESULTS

Maltase levels in inbred strains: Inbred strains carrying different active *MAL* loci in a common background were grown in maltose broth to log phase, and growth and maltase levels were monitored as described in MATERIALS AND METHODS. The results for three typical strains carrying either *MAL1*, *MAL2* or *MAL6*

are shown in Figure 1. The results are presented as differential growth curves, enzyme activity per milliliter of culture plotted against growth. The slopes of the differential growth curves represent the rate of accumulation of maltase per unit of growth.

The slopes of the differential curves shown in Figure 1 are 2.5, 5.4 and 2.7 for the strains carrying *MAL1*, *MAL2* and *MAL6*, respectively. These slopes indicate that the *MAL2* strain accumulates about twice as much maltase as the *MAL1* and *MAL2* strains. Since our "standard background" was similar to that of the *MAL2* strain, it seemed possible that other genes in the *MAL2* background, not the *MAL2* locus itself, might be responsible for the high enzyme levels in the

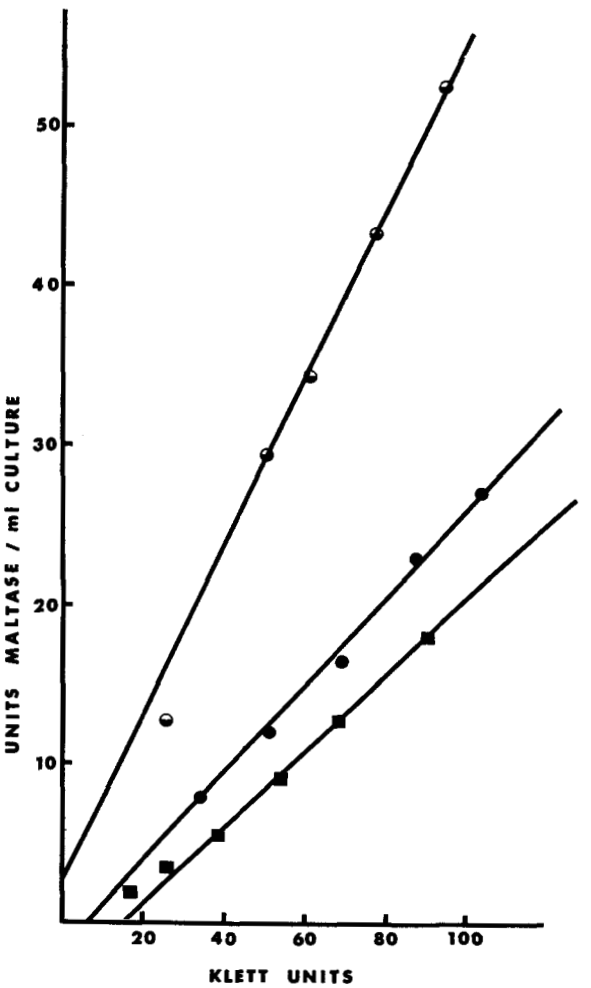

FIGURE 1.—Maltase synthesis in strains carrying different *MAL* loci. Haploid strains carrying *MAL1*, *MAL2* or *MAL6* were grown to log phase in maltose broth by Method A. Maltase activity and growth were monitored as described in MATERIALS AND METHODS. Symbols: ■ M88–10A (*MAL1*); half-filled circles M53-13B (*MAL2*); ● M85–79 (*MAL6*).

MAL2 strains. If this were correct, then inbreeding into the *MAL2* background should have increased the enzyme levels in the *MAL1* and *MAL6* strains. Therefore, enzyme levels were compared for segregants from each stage of inbreeding. The slopes of the differential growth curves are presented in Table 1.

No significant changes in enzyme levels were observed during inbreeding. All *MAL2* strains tested accumulated about twice as much maltase as the other *MAL* strains, and *MAL6* strains accumulated slightly more enzyme than the *MAL1* strains, as found previously (see Figure 1).

Segregation of enzyme levels: If the observed differences in enzyme levels are caused by the *MAL* loci themselves, then each *MAL* locus and the ability to produce a particular level of maltase should segregate together in a cross involving two *MAL* loci. Therefore inbred strains carrying *MAL1*, *MAL2* or *MAL6* were crossed in all three possible combinations (see Table 2), the diploids were sporulated and tetrads containing four maltose fermenting segregants (4:0 tetrads) were identified by tetrad analysis.

In a 4:0 tetrad, two of the segregants carry one of the two *MAL* loci segregating in the cross and the other two segregants carry the other *MAL* locus. Therefore the effects of the two *MAL* loci can be compared in all four products of a single meiosis, and any differences determined by the *MAL* loci should segregate 2:2. In tetrads containing only 2 or 3 maltose fermenting segregants (2:2 and 3:1 tetrads), some of the segregants do not carry an active *MAL* locus, and these do not provide information about the separate effects of the two loci. Therefore only segregants from 4:0 tetrads were examined further.

To determine if the maltase levels were actually segregating in the crosses, the rates of maltase accumulation were measured in the parent strains and in several complete 4:0 tetrads from each cross. Conditions were the same as in previous experiments, except that minimal medium was used instead of broth. The differential growth curves for a typical tetrad from M119 (*MAL1* × *MAL2*) are shown in Figure 2. The slopes of the differential curves for the parents and for two tetrads from each cross are given in Table 3.

In all three crosses examined, the enzyme levels segregated two "high" to two "low," as shown in Figure 2, although the difference between "high" and "low" varied from cross to cross. The difference was relatively large in the crosses involving *MAL2* and relatively small in M120 (*MAL1* × *MAL6*), as expected from the enzyme levels found in the parent strains. All enzyme levels were lower than expected on the basis of previous experiments, presumably because of the change from broth to minimal medium.

Co-segregation of enzyme levels and Mal *loci*: To determine if the 2:2 segregation of the enzyme levels corresponded to the segregation of the MAL loci, it was necessary to determine the *MAL* locus carried by each segregant from the 4:0 tetrads. Therefore, each segregant was crossed to one or two *MAL* tester strains carrying a single known *MAL* locus. The resulting diploids were sporulated and subjected to random-spore analysis, as described, and the proportion of haploid Mal$^-$ progeny resulting from each diploid was determined. The segregants from 7 or 8 4:0 tetrads of each cross (M118–M120) were analyzed in this way; the

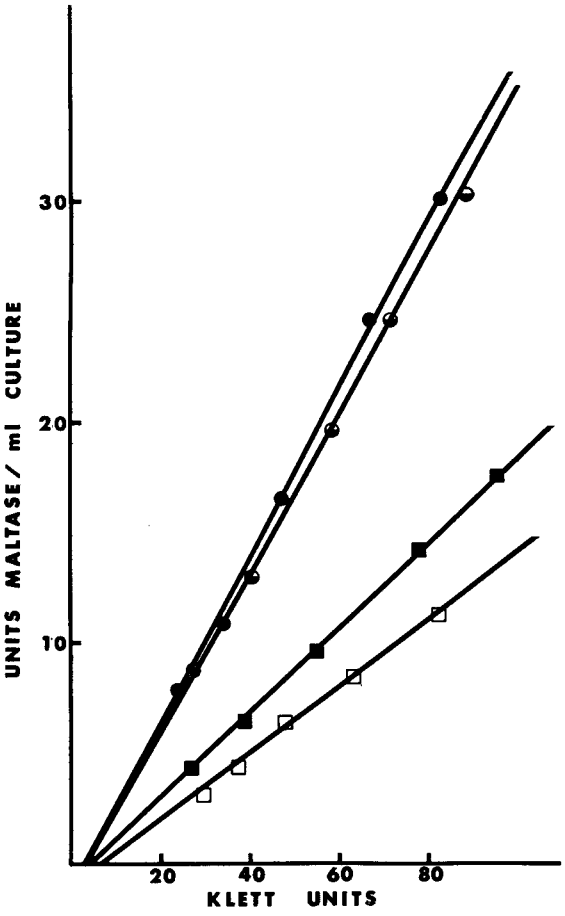

FIGURE 2.—Maltase synthesis in segregants from one tetrad of M119 (*MAL1* × *MAL2*). The 4 haploid segregants in tetrad 13 of cross M119 were grown to log phase in minimal medium containing 2% maltose by Method A. Maltase activity and growth were monitored as described in MATERIALS AND METHODS. Symbols: ● M119–13 (*MAL2*); half-filled circles M119–13B (*MAL2*); □ M119–13C (*MAL1*); ■ M119–13D (*MAL1*).

results for the segregants from two tetrads of each cross are summarized in Table 4. The table lists the segregants, the tester strains to which they were crossed and the number of Mal$^+$ and Mal$^-$ progeny recovered from sporulation of the resulting diploids. The expected number of Mal$^+$ and Mal$^-$ progeny is also given in the table. The *MAL* locus carried by each segregant was deduced from the proportion of Mal$^-$ progeny recovered, according to the following line of reasoning. If the tester strain and the segregant carried identical *MAL* loci, (*e.g.*, *MAL1*), a cross between them should generate a homozygous Mal$^+$ diploid (*e.g.*, *MAL1/MAL1*) and sporulation of the diploid should yield only Mal$^+$ progeny. If the tester and the segregant carried different *MAL* loci, (*e.g.*, *MAL1* and *MAL2*), a cross between them should generate a diploid heterozygous at both loci (*e.g.*, *MAL1/mal1*

TABLE 3

Maltase production in parents and segregants of crosses with two MAL *genes*

Strain	*MAL* locus*	$\frac{\Delta\text{maltase}\dagger}{\Delta\text{growth}}$ Observed	$\frac{\Delta\text{maltase}\dagger}{\Delta\text{growth}}$ Expected‡
parent 1A (M93–9C)	1	1.5	—
parent 1B (M93–19B)	1	1.7	—
parent 2 (M59–5A)	2	3.8§	—
parent 6 (M103–9C)	6	2.2§	—
Segregants from 2 × 6			
M118–3A	2	2.0	3.8
M118–3B	2	2.05	3.8
M118–3C	6	0.95	2.2
M118–3D	6	0.70	2.2
M118–20A	2	4.1	3.8
M118–20B	6	1.2	2.2
M118–20C	2	4.2	3.8
M118–20D	6	2.1	2.2
Segregants from 1A × 2			
M119–3A	1	1.8	1.5
M119–3B	2	4.2	3.8
M119–3C	1	2.4	1.5
M119–3D	2	4.6	3.8
M119–13A	2	3.7	3.8
M119–13B	2	3.6	3.8
M119–13C	1	1.6	1.5
M119–13D	1	2.0	1.5
Segregants from 1B × 6			
M120–3A	6	1.9	2.2
M120–3B	1	1.0	1.7
M120–3C	6	1.3	2.2
M120–3D	1	2.0	1.7
M120–5A	1	1.2	1.7
M120–5B	6	2.0	2.2
M120–5C	6	1.2	2.2
M120–5D	1	2.2	1.7

* *MAL* loci in segregants determined by backcrosses to standard strains, as shown in Table 4.
† Units as in Table 1. All cultures grown in minimal medium by Method A except M118 tetrad 20, which was grown by method B, as described in MATERIALS AND METHODS.
‡ Enzyme activity of parent strain with the same *MAL* locus as the segregant.
§ Average of two experiments.

MAL2/mal2) and sporulation of the diploid should yield 1/4 Mal$^-$ progeny. Therefore, if no Mal$^-$ progeny were recovered from the random-spore analysis, it was assumed that the *MAL* loci in the tester and the segregant were identical; if a significant number of Mal$^-$ progeny were recovered, it was assumed that the *MAL* locus in the segregant was different from the one used in the tester. Each segregant from M119 and M120 was crossed to two testers, one carrying each

TABLE 4

Identification of MAL *loci in segregants of crosses with two* MAL *genes*

Cross	Segregant	Presumed genotype	×*MAL1* tester			×*MAL2* tester			×*MAL6* tester		
			Total progeny tested	# Mal⁻		Total progeny tested	# Mal⁻		Total progeny tested	# Mal⁻	
				Found	Expected		Found	Expected		Found	Expected
M118	3A	*MAL2*				54	0	0			
	3B	*MAL2*				53	0	0	NT		
	3C	*MAL6*				55	12	14			
	3D	*MAL6*				52	15	13			
	20A	*MAL2*				49	0	0			
	20B	*MAL6*				46	13	10	NT		
	20C	*MAL2*				50	0	0			
	20D	*MAL6*				55	14	14			
M119	3A	*MAL1*	32	0	0	55	16	14			
	3B	*MAL2*	55	16	14	31	0	0			
	3C	*MAL1*	43	1	0	23	5	6			
	3D	*MAL2*	26	6	6	17	0	0			
	13A	*MAL2*	14	4	4	14	0	0			
	13B	*MAL2*	15	3	4	16	0	0			
	13C	*MAL1*	14	0	0	19	4	5			
	13D	*MAL1*	44	0	0	15	4	4			
M120	3A	*MAL6*	54	16	14				52	0	0
	3B	*MAL1*	41	0	0				52	15	13
	3C	*MAL6*	49	11	12				52	0	0
	3D	*MAL1*	49	0	0				55	12	14
	5A	*MAL1*	49	1	0				46	11	12
	5B	*MAL6*	53	13	13				51	0	0
	5C	*MAL6*	56	13	14				54	0	0
	5D	*MAL1*	56	0	0				54	15	14

MAL locus segregating in the original cross (M119 or M120). As the data in Table 4 show, the results with the two testers were complementary; if the cross to one tester produced a diploid that generated approximately 1/4 Mal⁻ progeny, the cross to the other tester produced a diploid that generated virtually none. These results indicated that it was possible to deduce the *MAL* locus carried by each segregant using only one tester; this was done for the segregants of M118. For all 4:0 tetrads tested from all three crosses, the *MAL* locus carried by each segregant could be unambigously assigned, and each *MAL* locus segregated 2:2 as expected.

In Table 3, the segregation of the *MAL* loci is compared with the segregation of maltase levels for two complete 4:0 tetrads from each cross. The average maltase levels of all *MAL1*, *MAL2* and *MAL6* segregants from each cross (and the levels in each parent) are presented in Table 5. The data presented in both tables demonstrate that the high level of maltase accumulation associated with the parent *MAL2* strain segregates with the *MAL2* locus in all 12 tetrads tested from M118 and M119. On the other hand, the small difference between the *MAL1* and *MAL6*

parent strains does not segregate with the *MAL* loci in the two tetrads tested from M120; the *MAL1* and *MAL6* segregants from all three crosses possess indistinguishable levels of maltase (Table 5).

From these results, it appears that the *MAL2* locus itself is responsible for the relatively high level of maltase in all of our *MAL2* strains. The *MAL1* and *MAL6* loci appear to allow a relatively low level of maltase accumulation that is the same for both loci. If the *MAL1* and *MAL6* loci allow accumulation of significantly different levels of maltase, the differences must be too small to detect, using our experimental conditions, or they must be obscured by the effects of other genes segregating in the cross.

Other crosses in this laboratory (data not shown) have indicated that genes other than the *MAL* loci can significantly affect the levels of maltase. These crosses were similar to M118 (*MAL2* × *MAL6*), but involved strains that were not as extensively inbred. In these crosses, the *MAL* loci segregated 2:2, but the enzyme levels did not. It was assumed that major unidentified regulatory loci were segregating in these crosses in addition to the *MAL* genes. The results of M118 (*MAL2* × *MAL6*) and M119 (*MAL1* × *MAL2*) indicate that similar major regulatory loci with large effects on the levels of maltase are not segregating in our standard background. However, minor loci with small effects could be segregating in M118–M120. If small differences in enzyme levels were caused by the segregation of these genes, their effects might be noticed in M120, but not in M118 and M119.

Induction in inbred strains: The results shown in Tables 1, 3 and 5 represent maltase levels in fully induced strains. Since there is considerable genetic evidence that the *MAL* loci are regulatory genes controlling the induction of maltase (see above), the kinetics of induction were examined in three strains carrying the three different *MAL* loci. The strains were pregrown to log phase in minimal medium without maltose. Maltose was added and growth and maltase levels were monitored as described. The induction curves are shown in Figure 3. It is clear from these curves that the three strains show different kinetics of induction; the *MAL2* strain produces maltase relatively quickly, while the *MAL1* strain pro-

TABLE 5

Average levels of maltase production in segregants of crosses with two MAL *genes*

Cross	*MAL* loci in cross	Δmaltase/Δgrowth* in parent strains			# Tetrads tested	Δmaltase/Δgrowth* in segregants†		
		MAL1	*MAL2*	*MAL6*		*MAL1*	*MAL2*	*MAL6*
M118	2 × 6	—	3.15	1.7	8	—	3.2±0.9	1.4±0.6
M119	1 × 2	1.5	4.4	—	4	1.8±0.5	3.9±0.4	—
M120	1 × 6	1.7	—	2.6	2	1.6±0.6	—	1.6±0.6
Avg. for all three crosses		1.6±0.1	3.8±0.9	2.2±0.6	14	1.8±0.5	3.4±0.8	1.4±0.6

* Units as in Table 1. All cultures grown in minimal medium by method A or B, as described in MATERIALS AND METHODS.

† *MAL* loci in segregants determined by backcrosses to standard strains, as shown in Table 4.

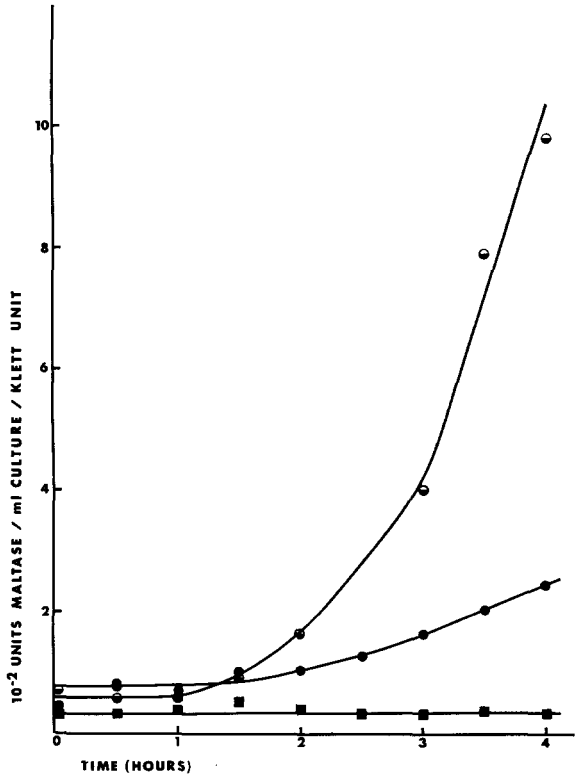

FIGURE 3.—Induction of maltase in strains carrying different *MAL* loci. Haploid strains carrying *MAL1*, *MAL2* or *MAL6* were grown on nonfermentable carbon sources. Maltose was added at time zero to start induction, and maltase activity and growth were monitored, as described in MATERIALS AND METHODS. Symbols: ■ M93-19B (*MAL1*); half-filled circles M59-5A (*MAL2*); ● M103-9C (*MAL6*).

duces maltase relatively slowly. To facilitate the comparison of induction curves such as those shown in Figure 3, the relative enzyme levels after four hours of induction were calculated. These are given in Table 6. The relative maltase levels in the three standard strains after 4 hours were 1, 33 and 8 in the *MAL1*, *MAL2* and *MAL6* strains, respectively.

Co-segregation of induction kinetics and MAL *loci*: To determine if the differences in induction were actually caused by the *MAL* loci, the kinetics were measured for the segregants of M118–M120. All segregants from 4:0 tetrads were pregrown without maltose, maltose was added and the cultures were grown in the presence of maltose (induced) for 4 to 5 hours. In the segregants from at least one tetrad from each cross, growth and maltase activity were monitored quantitatively throughout the period of induction, as in Figure 3. For the remaining tetrads, maltase activity was measured qualitatively after four or five hours, as described in MATERIALS AND METHODS. The results from the quantitative procedure are presented in Figure 4 and Table 6. Figure 4 shows the induction curves for a typical

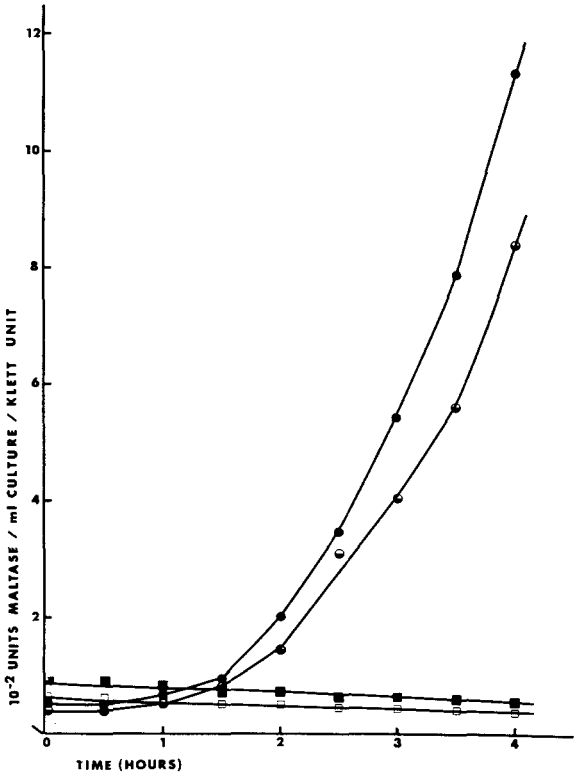

FIGURE 4.—Induction of maltase in segregants from one tetrad of M119 (*MAL1* × *MAL2*). The 4 haploid segregants in tetrad 13 of cross M119 were grown on nonfermentable carbon sources. Maltose was added at time zero to start induction and maltase activity and growth were monitored, as described in MATERIALS AND METHODS. Symbols: ● M119-13A (*MAL2*); half-filled circles M119-13B (*MAL2*); □ M119-13C (*MAL1*); ■ M119-13D (*MAL1*).

tetrad from M119 (*MAL*1 × *MAL2*). Table 6 lists the relative enzyme levels after four hours of induction for a typical tetrad for each cross (and for the parent strains). It is clear from the data in Table 6 (and Figure 4) that the differences in the kinetics of induction segregate with the *MAL* loci in the three tetrads examined quantitatively.

An additional 19 tetrads were tested by the qualitative procedure: seven more from M118 and 6 more each from M119 and M120. The differences in induction kinetics segregated 2:2 with the *MAL* loci in all 19 tetrads. In other words, the maltase level of each segregant (after 4 to 5 hours of induction) matched that of the parent carrying the same *MAL* locus. Therefore, it appears that the *MAL* loci themselves regulate the kinetics of induction (in our background) and that in our strains the rates of induction increase in the order *MAL2* > *MAL6* > *MAL1*.

DISCUSSION

The results presented in this paper show that strains carrying the three different *MAL* loci, *MAL1*, *MAL2* and *MAL6*, have different regulatory properties that

TABLE 6

Induction of maltase in parents and segregants of crosses with two MAL *genes*

Strain	Origin of strain	*MAL* locus*	Induced enzyme activity†	Relative induced enzyme activity‡	
				Observed	Expected§
M93-19B	parent	1	0.03	1.0	—
M59-5A	parent	2	0.98	32.7	—
M103-9C	parent	6	0.24	8.0	—
M118-3A	2 × 6	2	0.79	26.3	32.7
M118-3B	2 × 6	2	0.96	32.0	32.7
M118-3C	2 × 6	6	0.16	5.3	8.0
M118-3D	2 × 6	6	0.18	6.0	8.0
M119-13A	2 × 1	2	1.13	37.7	32.7
M119-13B	2 × 1	2	0.84	28.0	32.7
M119-13C	2 × 1	1	0.04	1.3	1.0
M119-13D	2 × 1	1	0.05	1.67	1.0
M120-5A	6 × 1	1	0.02	0.67	1.0
M120-5B	6 × 1	6	0.31	10.3	8.0
M120-5C	6 × 1	6	0.28	9.3	8.0
M120-5D	6 × 1	1	0.04	1.3	1.0

* *MAL* loci in segregants determined by backcrosses to standard strains as shown in Table 4.
† Units enzyme/ml × 10/Klett units after 4 hours of induction, determined from curves similar to those in Figure 4.
‡ Enzyme activity relative to the value for M93-19B (*MAL1* parent).
§ Relative enzyme activity of parent strain with the same *MAL* locus as the segregant.

are attributable to the *MAL* loci themselves. The properties shown here to be affected by the *MAL* loci are the kinetics of maltase induction and the level of maltase accumulation in fully induced cultures. In the strains tested, the relative rates of induction were *MAL2* > *MAL6* >> *MAL1*; the relative induced maltase levels were *MAL2* > *MAL1* ~ *MAL6*. The rates of induction and the induced maltase levels have been shown to be properties of the *MAL* loci by their cosegregation with the *MAL* genes in crosses involving either one (Table 1) or two different (Tables 3, 5 and 6) *MAL* loci.

KHAN, ZIMMERMAN and EATON (1973) demonstrated that the *MAL4* locus also regulates the level of maltase accumulation by examining the antigenic specificity and amount of maltase present in *MAL4* strains and in nonfermenting *mal4* mutants. The small amount of maltase in the mutants appeared to be identical in structure and function to that in the original Mal$^+$ strain, as expected if *MAL4* controls the amount of maltase synthesis, but not the primary structure of the enzyme.

Differences in the rates of maltase accumulation during induction and steady-state growth could reflect differences in the rates of maltase synthesis, degradation or both. It seems unlikely that degradation is significant because maltase activity is stable in the absence of protein synthesis (CHANDRA 1977) and after removal of the inducer (D.MOWSHOWITZ, unpublished data). If degradation is insignifi-

cant, then the results reported here indicate that the *MAL* loci control the rate of maltase synthesis both during induction and in the fully induced state.

The *MAL* loci could appear to control the accumulation of maltase because they are (or include) regulatory elements or because they are structural genes that encode enzymes that both metabolize maltose and regulate maltose metabolism. In either case, the differences observed here must be caused by differences in the *MAL* genes themselves (if they contain regulatory sites) or in their products (if they encode enzymes or regulatory proteins). If it is assumed that the *MAL* genes encode maltase and maltose permease, but not a regulatory protein, then the differences observed here must be explained on the basis of differences between the enzymes encoded by the various *MAL* loci. Attempts to find differences between the maltases in different strains have not been successful.

HALVORSON, WINDERMAN and GORMAN (1963) examined the maltase in strains carrying different *MAL* loci; no significant differences were found in the enzyme itself. Preliminary results from isoelectric focusing gels run in this laboratory have also failed to establish significant differences in maltase itself. All strains tested, both Mal^+ and Mal^-, produced a single, identical major maltase band and two minor maltase bands. Some additional minor α-glucosidase bands were found in some, but not all, strains tested (unpublished results of R. BLACK and L. L. SPIELMAN). Experiments are in progress to determine whether the differences in the minor bands are under the control of the *MAL* genes. Since the maltase in all the strains is indistinguishable, it seems highly unlikely that differences in the levels of maltase accumulation can be attributed to differences in the specific activity of maltase or in the autoregulatory properties of the enzyme itself.

It is possible that the differences in induction kinetics reported here could be caused by differences in the maltose permeases encoded by the different strains. If the *MAL1* permease were relatively inactive, compared to those in the *MAL2* and *MAL6* strains, then induction in the *MAL1* strains would be extremely slow, as observed. However, differences in permease activity should not affect the level of maltase accumulation after prolonged growth on maltose, so that differences in induced maltase levels are not explained by the assumption that the *MAL* loci encode maltose permease that differs from strain to strain.

If the *MAL* loci contain *cis*-acting regulatory sites or encode *trans*-acting regulatory proteins, then the results reported here are readily explained by variations in the regulatory sites or in the regulatory proteins encoded by the different *MAL* loci. These regulatory sites and/or proteins could control maltase production in response to maltose and possibly also mediate catabolite repression of maltase production in response to glucose (ZIMMERMAN and EATON 1974). Sensitivity of the regulatory system to maltose could regulate induction kinetics, while sensitivity to both maltose and glucose (which is generated from maltose by the action of maltose) could regulate the steady state levels of maltose accumulation.

Mutational analysis of the *MAL2* locus by ZIMMERMAN and EATON (1974) and the *MAL6* locus by TEN BERGE and associates demonstrated in a different way that *MAL2* and *MAL6* must be (or include) regulatory loci. These workers showed that uninducible and constitutive mutations map in the *MAL2* (ZIMMER-

MAN and EATON 1974) and *MAL6* loci (TEN BERGE, ZOUTEWELLE and VAN DE POLL 1973; TEN BERGE *et al.* 1973) and that the enzyme maltase itself is unaltered in *mal6* strains that are uninducible or temperature sensitive for induction (TEN BERGE, ZOUTEWELLE and VAN DE POLL 1973). EATON and ZIMMERMAN obtained strains that contain temperature-sensitive maltase, but the genes responsible do not appear to be linked to the *MAL* gene in the *MAL3* parent strain (EATON and ZIMMERMAN 1976) or to other *MAL* genes (N. R. EATON, personal communication).

All these results are consistent with the proposal that the five *MAL* loci (*MAL1*, *MAL2*, *MAL3*, *MAL4* and *MAL6*) contain regulatory genes controlling the induction of the maltose fermentation enzymes. Since the sites of the structural genes for maltase, maltose permease, etc., are not known, it is possible that the *MAL* loci also include the structural genes for these enzymes. It has been suggested that each *MAL* locus contains at least one structural gene for maltase (as well as a regulatory region) to explain the absence of maltase structural mutations and the existence of complementing mutations in the same or different *MAL* loci (OSHIMA 1967; NAUMOV 1971).

The alleles of *MAL1*, *MAL2* and *MAL6* used in this work have different effects on the regulation of maltase accumulation. These differences have allowed us to demonstrate that the *MAL* loci themselves regulate maltase accumulation. However, it is not known whether all active *MAL1* (or *MAL2* or *MAL6*) alleles are identical, so that it is possible thatt he properties attributed here to each *MAL* locus are really attributable only to a particular allele of that locus. In other words, it is possible that there is as much variation between the different alleles of any one *MAL* locus as that observed here between the alleles of three different *MAL* loci. If this is so, not all strains of Saccharomyces carrying *MAL1*, *MAL2* or *MAL6* will exhibit the same regulatory properties as those described here. Although this does not affect the argument that the *MAL* loci serve a regulatory function, it does effect what is to be expected from a "standard" laboratory strain carrying *MAL1*, *MAL2* or *MAL6*.

Whether or not all active *MAL1* (or *MAL2* or *MAL6*) loci are identical, the alleles carried by the stains described here have been shown to exert different effects on the regulation of maltase accumulation. Therefore experiments are in progress to confirm their effects on the timing of maltase synthesis during the cell cycle (TAURO and HALVORSON 1966).

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