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Upstream Regulatory Regions Controlling the Expression of the Yeast Maltase Gene

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The expression of the maltase (*MALS*) and the maltose permease (*MALT*) genes in *Saccharomyces* species is coregulated at the transcriptional level; they are coordinately induced by maltose in the presence of a positively acting regulatory (*MALR*) gene and carbon catabolite repressed by glucose. We generated a series of deletions in the upstream region of the *MAL6S* gene to examine the regulatory elements in detail. The results showed that inducible expression by maltose was lost when the region between 320 and 380 base pairs upstream of the translation initiation codon was deleted. This region contained an imperfect inverted repeat sequence (−361 to −327) or four copies of short direct repeats that might serve as components of the upstream activation site (UAS_M) for the maltase gene, or both. When a stretch of T-rich sequence (−253 to −237) was deleted, the susceptibility of the maltase gene to carbon catabolite repression was affected.

Genetic analysis has shown that any one of the family of at least five unlinked polymeric *MAL* loci (*MAL1* to *MAL4* and *MAL6*) confers on *Saccharomyces cerevisiae* the ability to grow on maltose as a sole carbon source (2). Not only are the *MAL* loci functionally equivalent, but they show extensive sequence homology (4, 27; T. Chow, unpublished data). The structural genes (*MAL6S* and *MAL6T*) encode maltase and maltose permease, respectively; together with a positively acting regulatory gene (*MAL6R*), they have been shown to constitute a cluster of three genes at the *MAL6* locus (4, 5, 30). The two divergently transcribed structural genes are coordinately induced by maltose in the presence of the *MALR* product and are repressed by glucose at the transcriptional level (5, 10, 30).

Using cloned and subcloned fragments of the *MAL6* locus from *S. carlsbergensis* (9) and in vitro deletion analysis, we were able to examine more closely the regulation of maltose fermentation in yeast. A portion of the *MAL6* locus was sequenced, including the entire *MAL6S* gene, part of the *MAL6T* gene, and the intergenic (IG) region between them (19). The transcription initiation sites of the divergently transcribed genes were also determined. Our results showed that the ATG translation initiation codons of the *MAL6S* and *MAL6T* genes are 884 base pairs (bp) apart (19).

In the yeast *S. cerevisiae*, it has been shown that the regulation of transcription of several structural genes is mediated via a *cis*-acting upstream activation sequence (UAS) as well as by TATA elements and transcription initiation sites (13, 15, 38, 39, 42). The UASs function in response to various stimuli and can act at long and variable distances from the RNA polymerase II-mediated transcription initiation site. Examples of well-characterized UASs include those of the *GAL1-10* gene cluster (16, 22, 43), the *HIS3* and *HIS4* loci (18, 39), and the *CYC1* gene (14, 15). When galactose is utilized as a carbon source in yeast, a cluster of closely linked structural genes (*GAL1*, *GAL7*, and *GAL10*) are coordinately induced about 1,000-fold, regulated at the level of transcription (20, 37). It was later revealed that the increased transcription was mediated by binding of a positively acting regulatory gene (*GAL4*) product to a UAS_G region that could act in either orientation upstream of these structural genes (11, 22, 43). Some analogies are found between the *GAL* genes and the *MAL* loci in terms of structural arrangement as well as mode of regulation: (i) the structural genes in both cases are induced by the respective carbon source and carbon catabolite repressed by glucose; (ii) both systems require a *trans*-acting positive regulatory gene for their induction; and (iii) both sets of structural genes (*GAL1* and *GAL10* and *MAL6S* and *MAL6T*) are located several hundred base pairs apart and are divergently transcribed.

In this report we delimit the upstream control sequences of the *MAL6S* gene. Specifically, we have narrowed down the *cis*-acting control sequences required for the induction of the *MAL6S* gene; they appear to be the binding site for a protein(s) dependent on the expression of the regulatory gene (*MAL6R*). Furthermore, the significance of a stretch of T-rich sequence, detected between UAS_M and the transcription initiation site, that appears to be either the site of carbon catabolite repression or involved in the basal level expression of the maltase gene, is also discussed.

MATERIALS AND METHODS

Strains and growth conditions. *Escherichia coli* HB101 and RR101 (25) were grown on L-broth plus antibiotics and routinely used for the cloning and isolation of plasmid vectors. *E. coli* JM105 (31) was grown on 2× YT-broth and was used for the propagation of M13 phages for DNA sequencing to determine the endpoints of deletions. The compositions of L-broth and YT-broth used are those described by Miller (28). The *S. cerevisiae* strains used are listed in Table 1. Yeast cells were grown on either YP or synthetic complete (SC) medium lacking appropriate requirements when used to maintain plasmids, supplemented with carbon sources as described in Results and the figure legends. Cell strains harboring plasmids with deleted *MAL6S* 5′-flanking sequences were grown on SC medium lacking uracil.

Construction of deletion mutations in the intergenic region. The first series of sequential 5′ deletions (see Fig. 1 and 2) upstream of the *MAL6S* structural gene were generated as follows. Plasmid pCH134, which contains the entire *MAL6S* coding region, most of the intergenic region as far as the *MAL6T* translation initiation codon, and the 3′-flanking

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TABLE 1. *S. cerevisiae* strains used in this work

Strain	Genotype	Source or reference
JM2763-14	*MATa leu2-3 leu2-112 ura3-52 MAL1*	5
TCY70	*MATa ura3-52 MAL1S::LEU2*	5
TCY137	*MATa ura3-52 MAL1R::LEU2*	This laboratory
JM1991	*MATa his4 leu2-3 MAL1* (pMR42)[a]	This laboratory

[a] Plasmid pMR42 contains the *MAL6R* gene on multicopy plasmid YEp13 (5).

region of the *MAL6S* gene, was constructed in our laboratory. About 5 μg of this plasmid was first digested to completion with endonuclease *Bam*HI and then placed in buffer containing 20 mM Tris (pH 8.0), 100 mM NaCl, 12 mM $CaCl_2$, and 1 mM EDTA in a total volume of 50 μl. One unit of nuclease Bal 31 (International Biotechnologies, Inc.; slow and fast mixture) was added, the mixture was incubated at 37°C, and 10-μl portions were removed every 10 min. Following ethanol precipitation, unphosphorylated *Bam*HI linkers (12-mer; New England Biolabs) were ligated to the deleted ends as described by Lathe et al. (24). Ligated plasmids were transformed into *E. coli* HB101, and the approximate sizes of the deletions were determined by sizing *Bam*HI- and *Bgl*II-generated plasmid fragments. DNA fragments generated by *Bam*HI and *Hin*dIII double digestion of promising clones were isolated from the sizing gel and cloned into phage M13mp18 to determine the exact deletion endpoints by chain termination DNA sequencing (33). These fragments were then ligated to the yeast shuttle plasmid vector pCH250 (see Results and Fig. 1) doubly digested with *Bam*HI and *Hin*dIII to generate the deletion mutations upstream of *MAL6S*, designated pSD1 through pSD6 (see Fig. 2).

Another series of internal deletion mutations in the intergenic region were constructed by combining additional far-upstream sequences, generated by exonuclease III treatment, with deletion mutants pSD4 through pSD6 described above. Plasmid pCH134, cut with *Bgl*II within the *MAL6S* coding region, was used for the exonuclease III digestion. Treatment with exonuclease III (New England Biolabs) was carried out by the method of Guo and Wu (17) with slight modification in the salt concentration. The reaction mixture was incubated at room temperature for up to 1 h, with 20-μl portions removed every 5 min, and heat-inactivated at 70°C for 10 min. Nuclease S1 (280 μl; 200 U/ml of buffer) was added to each portion and incubated at 37°C for 10 min to remove the single-strand termini left by the exonuclease III treatment. After phenol-chloroform extraction and ethanol precipitation, the treated plasmids were repaired by Klenow polymerase as described by Maniatis et al. (25). Unphosphorylated *Bgl*II linkers (10-mer; New England Biolabs) were ligated to the deleted ends as described above. Monitoring the approximate deletion size, sequencing promising deletions, and subsequent recloning into the plasmids pSD4 through pSD6 cut with *Bam*HI were carried out as described for the first set of deletions. Final constructions for the internal deletions in the upstream region are shown in Fig. 3.

These plasmid constructions were transformed into *S. cerevisiae* TCY70 (Table 1) by the Li_2SO_4 procedure of Ito et al. (21). Ura^+ transformants were selected and used for subsequent *MAL6S* expression by analyzing the maltase activities and steady-state maltase-specific mRNA levels.

Assay for maltase specific activity. Maltase specific activities were measured by assaying the rate of release of *p*-nitrophenol from *p*-nitrophenyl-α-D-glucopyranoside (pNPG) by the method described by Goldenthal et al. (12) and Dubin et al. (7). Based on the comparison of the pNPGase, maltase, and α-methylglucosidase, which can also cleave pNPG, it was clear that the pNPGase level found in the yeast cell extracts is an accurate measure of the amount of maltase present in the cell (7).

Slot blot hybridization analysis for maltase-specific mRNA levels. Total RNA was isolated from transformants as well as control strains grown on medium supplemented with different carbon sources by the method of Elion and Warner (8). Five micrograms of total RNA from each strain was dissolved in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and applied to the nitrocellulose membrane in a Minifold II apparatus (Schleicher & Schuell) with applied vacuum. Subsequent baking of the membrane and hybridization were performed as described by Elion and Warner (8). A cloned internal fragment from the *MAL6S* coding region, excised by *Bgl*II, was nick translated and used as the hybridization probe (25).

Gel retardation study for DNA-binding protein. Cell extracts were prepared from the yeast strains TCY137, JM2763-14, and JM1991 (Table 1) by the method of Bram and Kornberg (3). DNA fragments were isolated from pSD3 and pSD5 by digesting with *Bam*HI and *Bgl*II and 5′-end labeled with Klenow polymerase (25). The binding reaction and subsequent electrophoresis were carried out by the methods described by Cohen et al. (6) and Solomon et al. (36), except that 25 μg of poly(dI-dC) was used in each binding reaction as competitive DNA instead of *E. coli* DNA to reduce nonspecific DNA-protein binding.

RESULTS

Construction of deletions in the 5′ regulatory region of *MAL6S*. To define *cis*-acting sequences that regulate expression of the *MAL6S* gene, we initially constructed a series of sequential deletions in its 5′ upstream region. Figure 1 summarizes the strategy for generating such upstream deletions (pSD1 through pSD6), of internal deletions (pSH21 through pSH36) in the IG region, and the subsequent cloning of these deletion constructs into a centromere (*CEN*)-based plasmid vector. The centromeric plasmid pCH250 is a deletion-modified version of the *CEN4*-containing yeast vector YCp50, originally described by Kuo and Campbell (23). The plasmid was modified by removing the promoter region of the Tet^r gene as well as part of its coding region so that its strong promoter would not interfere with expression of the cloned yeast sequences. Upstream deletions were constructed in the region −168 to −674 by digesting with exonuclease Bal 31. Internal deletions of variable length in the region −168 to −415 were generated by combining the exonuclease III-generated sequences farther upstream with the deletion mutants pSD4 through pSD6; the method used was similar to the linker-scanning method described by McKnight and Kingsbury (26). Both series of deletion mutations retained the entire *MAL6S* coding region, its 3′-flanking sequences, and the region between the TATA box and the *MAL6S* transcription initiation site (Fig. 2 and 3).

Identification of the upstream *cis*-acting regulatory sequences of the *MAL6S* gene. To map the apparent 5′ boundary of potential upstream *MAL6S* regulatory sequences, we initially analyzed the expression of a series of deletion mutations extending for variable distances from position −674 toward the translation initiation codon of *MAL6S*. The endpoints of these deletion mutations are indicated in Fig. 2.

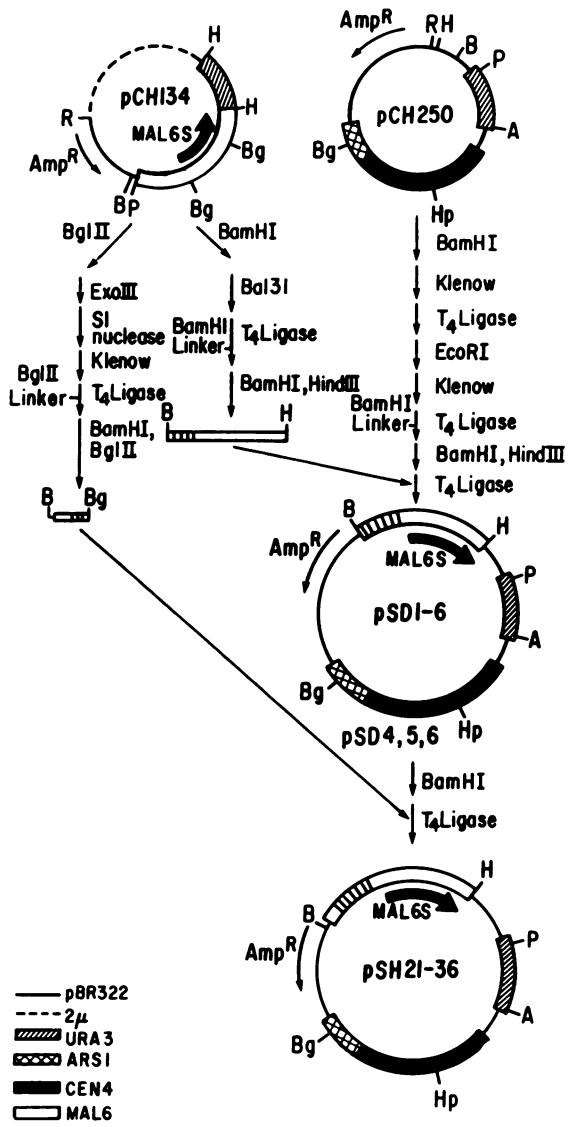

FIG. 1. Construction of deletion mutations in the upstream region of the *MAL6S* gene. Two series of plasmids (pSD1 through pSD6 and pSH21 through pSH36) containing various upstream deletions were constructed as described in Materials and Methods. Restriction sites: A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; R, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*II; P, *Pst*I. Plasmid pCH250 is the deletion-modified version of YCp50 (23) as described in Results. *URA3*, 2μ, *ARS1*, *CEN4*, and *MAL6* are yeast sequences. ExoIII, Exonuclease III.

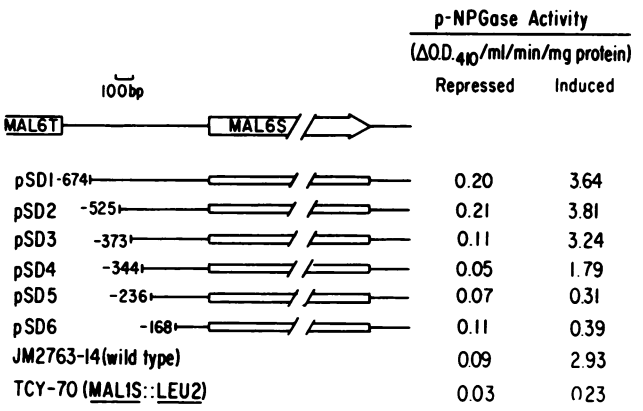

FIG. 2. Upstream deletions in the IG region and their effect on maltase-specific activity. Coding regions for the *MAL6S* and *MAL6T* genes are shown by the large open arrows. Each deletion endpoint in plasmids pSD1 through pSD6 is indicated by numbers representing the upstream distance (in base pairs) from the ATG translation initiation codon of the *MAL6S* gene. Maltase specific activities were assayed as pNPGase and are shown on the right. Enzyme assays were performed in duplicate and values were normalized. Repressed values are the pNPGase activities from cells grown on 2% glucose; induced values are those from cells grown on 2% raffinose plus 2% maltose, as described in Materials and Methods.

The sequences are numbered from the first nucleotide of the translation initiation codon ATG (19). Expression of the maltase gene was monitored by assaying pNPGase activity, which quantitatively reflects the level of maltase activity present in yeast cell extracts (7, 12, 29).

Maltase genes with upstream deletions extending from position −674 to position −373 (pSD1 through pSD3) were induced about 30-fold by maltose, approximating the level reached by induced control cells carrying the intact genomic *MAL1S* gene (Fig. 2). Raffinose, instead of glycerol and ethanol, was added as a nonrepressing carbon source in the inducing conditions since the recipient strain (TCY70) grew poorly on nonfermentable carbon sources. The level of expression from the cloned *MAL6S* gene was expected since the centromere-based plasmid used for cloning it and the adjoining upstream deletion constructs is present in only one or two copies per cell (1, 23). In contrast, the induction of *MAL6S* adjoining deletions extending from position −344 and beyond was reduced or almost totally abolished. When cells harboring plasmids with these upstream deletions were grown under repressing conditions (i.e., on glucose), the levels of pNPGase activity did not appear to be affected significantly. Those data place the apparent 5′ boundary of the *cis*-acting regulatory region between positions −373 and −344 or beyond.

When cells harboring the series of internal deletion mutations were grown on various carbon sources, two different responses were detected depending on the upstream regions deleted (Fig. 3). The *MAL6S* genes with internal deletions extending from positions −168 to −329 in the IG region (pSH21 through pSH26) were induced to a level comparable to the normal induced level of a strain carrying a single *MAL1S* genomic copy. However, when the deletion was extended farther upstream to position −380 (pSH36), inducibility by maltose was lost. Another series of internal deletion mutants extending from positions −236 to −380 (pSH27 through pSH31) exhibited the same trend, with only the deletion mutant extending from position −236 to −380 (pSH31) losing the ability to be induced by maltose. In addition, two constructions, each with a small region of overlap (pSH32 and pSH33) as well as small deletions from −344 to −415 (pSH34 and pSH35), retained the ability to express the structural gene under inducing conditions (Fig. 3). These results place the apparent 3′ boundary of the regulatory region 334 bp upstream of the ATG translation initiation codon. From these results, we may conclude that the region between 334 and 380 bp upstream from the translation initiation codon is important for induction of the maltase gene.

Another interesting finding was that when cells harboring plamids with various deletions were grown under repressing conditions, either in glucose alone or in glucose plus malt-

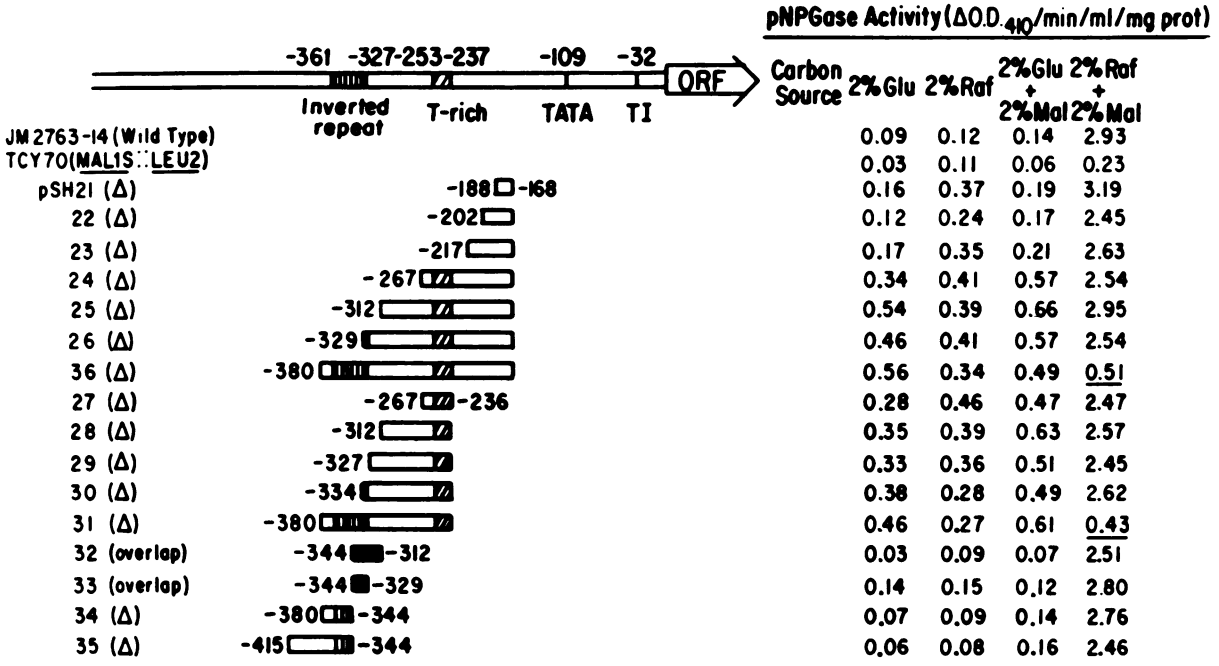

FIG. 3. Internal deletions in the IG region and their effect on maltase specific activity. The open reading frame (ORF) within the open arrow is the coding region for the *MAL6S* gene. The extents of the deletions are delineated by the number of base pairs from the *MAL6S* ATG codon, as in the legend to Fig. 2. In pSH32 and pSH33, there is an overlap in the sequence between −344 and −312 and −344 and −329, respectively (Fig. 2). TI on the top bar indicates the transcription initiation site of *MAL6S* (19). The T-rich sequence and the inverted repeat are shown as distinct blocks and are discussed in the Results and Discussion sections. Maltase specific activities of cells harboring various plasmids, grown on various carbon sources, are listed. Enzyme assays were performed as described in the legend to Fig. 2. Glu, Glucose; Raf, raffinose; Mal, maltose.

ose, some of the mutant constructs (pSH24 through pSH31 and pSH36) showed up to fivefold-higher levels of pNPGase activity than did repressed cells carrying the wild-type *MAL1S* gene. A pyrimidine-rich sequence with a high T content (15 of 17 bases) was found 237 to 253 bp upstream from the maltase translation initiation codon. All of the above-mentioned deletions that showed higher repressed levels than the wild type lacked this T-rich sequence. However, when these mutants were grown on the nonrepressing carbon source raffinose, the levels of pNPGase activity did not allow us to distinguish between the presence and absence of the T-rich sequence. Thus, two promoter elements seem to be present upstream of the *MAL6S* TATA sequence: a UAS_M regulatory sequence that responds to maltose induction and a T-rich sequence that acts at a lower level in response to glucose carbon catabolite repression.

Maltase-specific RNA levels in the deletion mutants. To complement the pNPGase activity assays, specific mRNA levels were studied. Total RNA was isolated from cells harboring the plasmids with the internal deletion constructs described earlier, which were grown on different carbon sources. The steady-state maltase-specific mRNA levels were monitored by slot blot hybridization (Fig. 4). The deleted sequences were found to affect the maltase-specific mRNA levels and enzyme activities in a parallel manner. These results confirm the previous findings that the regulation of maltase gene expression is exerted at the transcriptional level (10, 30).

Gel retardation studies for protein binding to a specific DNA region. To examine the possibility that the transcription of the *MAL6S* gene is regulated via the binding of a regulatory gene product to a *cis*-acting regulatory element, we performed DNA gel retardation studies. When a 387-bp DNA fragment (extending from +151 to −236) that did not contain the UAS_M defined by deletion analysis was added to cell extracts prepared from a strain carrying an integratively disrupted regulatory gene (*MAL1R::URA3*) (Fig. 5A, lane 2), a *MAL1R* strain (lane 3), and a strain carrying the

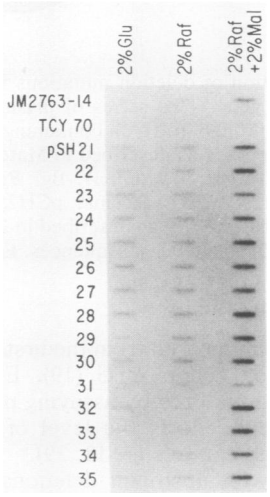

FIG. 4. Effect of deletions on the steady-state maltase-specific mRNA levels. Five micrograms of total cellular RNA from the deletion mutants was fixed on a nitrocellulose membrane and hybridized with a ^{32}P-labeled internal *MAL6S* gene fragment probe as described in Materials and Methods. Plasmids pSH21 through pSH35 carry the deletion constructs described in the legend to Fig. 3. Heading each column are the carbon sources on which the cells were grown. See Fig. 3 legend for abbreviations.

MAL6R gene on a multicopy plasmid (lane 4), no detectable retardation in the electrophoretic mobility of the DNA was found. Lane 1 in the figure represents the control labeled DNA fragment without any added extract. However, when a 524-bp DNA fragment (+151 to −373) that did contain the region essential for the induction of the maltase gene was subjected to the same set of DNA-protein binding experiments (Fig. 5B), retardation of DNA mobility on the gel was readily detected with cellular extracts prepared from strains expressing the regulatory gene (*MALR*) (lanes 3 and 4). Retardation in electrophoretic mobility is likely due to the binding of the regulatory gene product to a specific region of the DNA; cellular extract prepared from the strain that lacked the regulatory function was unable to retard the mobility of the same DNA fragment (Fig. 5B, lane 2). The presence of the inducer maltose in the reaction mixture did not influence the binding capacity (data not shown). From the results, one might infer that the *MALR* regulatory protein, previously shown to be required for the induction of the maltase and maltose permease genes (5, 10), binds (or controls the binding of other proteins) to a *cis*-acting upstream regulatory element, even in the absence of maltose.

DISCUSSION

A number of *cis*-acting upstream regulatory elements affecting yeast gene expression have been defined by analyzing the phenotypes of a set of deletion mutations that successively remove flanking DNA sequences of structural genes (42). Generally, deletions up to a certain point retain the phenotype essentially indistinguishable from the wild type, whereas deletions beyond a critical nucleotide sequence significantly reduce the expression of that particular gene below the normal level. This defines both the minimum

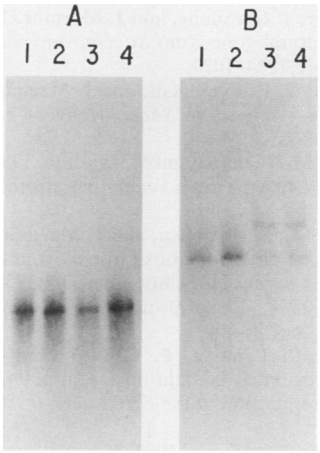

FIG. 5. Gel retardation study to detect DNA-binding proteins. Cell extracts from three different strains were mixed with 5′-end-labeled DNA fragments at room temperature for 20 min and electrophoresed on a low-ionic-strength 5% acrylamide gel (6). The gel was dried and subjected to autoradiography. (A) The 3′-end-labeled 387-bp DNA fragment (from positions +151 to −236) was used for one series of protein-binding assays. This DNA fragment does not contain the UAS_M, as described in the Results and Discussion sections. Lane 1, Labeled DNA alone; lanes 2, 3, and 4, binding reaction product with cell extracts from strains TCY137, JM2763-14, and JM1991 (see Table 1), respectively. (B) In this experiment, the 524-bp DNA fragment (from positions +151 to −373) which contains the UAS_M was used for the binding assays. Lane 1, DNA fragment alone, 5′-end labeled by Klenow polymerase; lanes 2, 3, and 4, same as in panel A.

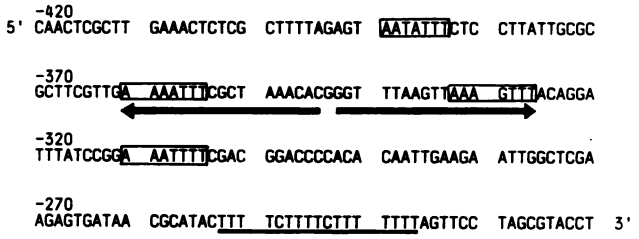

FIG. 6. Nucleotide sequence of the region required for the regulation of *MAL6S* gene expression. The nucleotide sequences are numbered from the ATG translation initiation codon of *MAL6S* (19). The inverted repeat structure is indicated by solid arrows below the nucleotide sequence. The four copies of the 7-bp short direct repeat sequence are boxed. Underlined is the 17-bp T-rich pyrimidine sequence described in the Results and Discussion sections.

contiguous region and the critical DNA sequences necessary for wild-type expression, even though such an analysis may not precisely delineate the upstream promoter elements. Previous studies on maltose fermentation in *S. cerevisiae* showed that a functional, positively acting regulatory gene (*MALR*) is required for the induction by maltose of two structural genes, *MALS*, encoding maltase, and *MALT*, encoding maltose permease (5, 30). At the *MAL6* locus, *MAL6S* and *MAL6T* are divergently transcribed from transcription initiation sites that are 785 bp apart (19). The intergenic region of the two genes, as in the case of *GAL1* and *GAL10* (16, 22), is likely to contain the *cis*-acting regulatory site(s) for the basal, induced, and carbon catabolite-repressed expression of *MAL6S* and *MAL6T*.

In this report we have delimited a *cis*-acting control element of the *MAL6S* gene of *S. cerevisiae*. The results demonstrate that a region 334 to 380 bp upstream of the *MAL6S* translation initiation codon contains the site responsible for transcriptional activation; it is required together with the *trans*-acting, positive regulatory gene product and maltose to induce maltase. A series of upstream as well as internal deletions flanking the structural gene *MAL6S* (Fig. 1, 2, and 3) were generated. The *MAL6S* coding region and the accompanying deletions in its 5′-flanking region were then inserted into a yeast centromeric vector and transformed into a yeast strain carrying a null mutation in *mal1S*. The transformants were isolated and analyzed for maltase activity as well as for their steady-state maltase-specific mRNA levels after growth on various carbon sources. Deletion of the region from 334 to 380 bp (pSH31) upstream of the translation initiation codon was clearly associated with the loss of inducible expression of the maltase gene. This was also accompanied by a low level of maltase-specific mRNAs in clone pSH31 (Fig. 4).

Examination of the pertinent DNA sequences responding to maltose induction revealed a large inverted repeat sequence within and neighboring the region that is necessary for the induction of the maltase gene. It extends from −327 to −361, with 22 of 34 bases paired as shown by the solid arrows in Fig. 6. Moreover, four copies of a 7-bp direct repeat sequence with the consensus sequence AAANTTT (boxed sequence) were found within and around this inverted repeat structure (Fig. 6). Several yeast UASs have been reported to contain one or more short inverted repeat sequences. Good examples are the *ADH2* gene (35) and the *GAL7, GAL1-10* gene cluster (16, 22, 43). In *CYC1* (14, 15) and *SUC2* (34), the UASs contain several copies of short direct repeats. Whether this inverted repeat sequence or the short direct repeats found in the upstream activation site

(UAS_M) for the *MAL6S* gene have any significance in terms of direct interaction with the regulatory protein and response to maltose remains to be investigated. The positions of four direct repeat sequences and the fact that the endpoint of one of the upstream deletion mutants, pSD4 (Fig. 2), fell exactly in the middle of the inverted repeat sequence at position −344 and showed about half the normal induced level of the maltase expression suggest that these structures may be involved in the induction of the maltase gene.

Results of the gel retardation study (Fig. 5) further support the proposal that the regulation of *MAL6S* gene expression is mediated via the binding of the positively acting regulatory gene product (or another protein dependent on *MAL6R* expression) to the promoter element UAS_M. More precise identification of the dimensions of the UAS_M will require additional deletion mapping studies, oligonucleotide-directed mutagenesis, synthesis of wild-type or altered oligonucleotides to replace the natural sequences, and DNA footprinting or methylation protection studies in the presence of specific DNA-binding proteins. Also, comparison of the sequences in the *MAL6* IG region to those of other *MAL* loci, such as *MAL1*, should provide additional information since the *MAL6R* product has been shown to act in *trans* to regulate the expression of the structural genes at the *MAL1* locus (5). It still remains to be determined whether the *MAL6S* UAS_M also plays a role in the induction of the *MAL6T* gene or whether a separate *cis*-acting element exists for this coregulated but divergently transcribed gene. This might best be answered by generating a gene fusion of *MAL6T* with β-galactosidase to provide a convenient assay. The same or a more detailed set of deletions in the 5′-flanking region of such a fusion would allow one to carry out an analysis similar to that for *MAL6S*.

A more cautious interpretation is required with regard to the function of the stretches of T-rich sequence (Fig. 6), found at positions 237 to 253 upstream of the *MAL6S* translation initiation codon (Fig. 3), in the expression of the maltase gene. When this 17-bp T-rich sequence was retained in mutant constructions (pSH21 to pSH23 and pSH32 to pSH35), the levels of maltase expression under repressed conditions were indistinguishable from that of wild type. However, the glucose-repressed level of pNPGase activities increased up to three to five times in the absence of the T-rich sequence. These results imply that this T-rich block may be one of several sites involved in the carbon catabolite repression of the *MAL6S* gene by glucose. Mutants that contain a long stretch of poly(dA-dT) homopolymer upstream of the *ADR2* gene have been shown to exhibit high-level constitutive expression of the gene (32). Also, naturally occurring poly(dA-dT) sequence, almost identical to the T-rich sequences found in the *MAL6S* upstream region, has been shown to act in a bidirectional manner as a component of the upstream promoter elements for the constitutive basal-level transcription of some genes in *S. cerevisiae* (40, 41). In that case, however, there is a slight difference in defining constitutive basal-level expression and catabolite repression since the genes examined, *PET56*, *HIS3*, and *DED1*, are not directly involved in carbon utilization. We have operationally defined the basal level of expression as the maltase activity present in cells grown on a nonrepressing, noninducing carbon source such as raffinose (Fig. 3). Our results favor the model that the T-rich stretch may be responsible in part for the carbon catabolite repression by glucose rather than the basal constitutive-level expression of the *MAL6S* gene. The possibility that this T-rich sequence may also be acting bidirectionally by participating in catabolite repression of the *MAL6T* gene is not excluded since no such large poly(dA-dT) sequence was detected in the region between the two structural genes.

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