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Regulation of Maltase Synthesis in *Saccharomyces carlsbergensis*

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The induction of maltase (EC 3.2.1.20) by its inducer maltose in a strain of the yeast *Saccharomyces carlsbergensis* carrying a functional *MAL* locus is regulated at the level of transcription. Preceding the synthesis of increased levels of maltase is the de novo synthesis of maltase-specific RNA sequences. This was detected by determining the level of maltase mRNA by DNA-RNA hybridizations by using a maltase structural gene DNA sequence probe and by assaying functional maltase mRNA by in vitro RNA-directed synthesis of immunologically reactive maltase. Once maltase has accumulated, late in induction, further synthesis of the enzyme is inhibited, as reflected by reduced levels of the mRNA that encodes maltase.

The yeast *Saccharomyces cerevisiae* and the closely related species *Saccharomyces carlsbergensis* synthesize a maltose-inducible and glucose-repressible α-glucosidase or maltase (EC 3.2.1.20). This intracellular enzyme is a monomer of 63,000 daltons and has been purified to homogeneity and characterized (14). The study of inducible maltase synthesis in yeasts offers several advantages, including ease of purification and assay of the enzyme. The recent availability (5) of the cloned maltase structural gene provides a probe for studying transcriptional regulation. The replacement of glucose by maltose in the growth medium results in the coordinate induction of maltase and a specific maltose transport system (2). This process is thought to involve the expression of at least three genes, a regulatory gene, the maltose permease, and the maltase structural genes (21). This report describes the sequence of biosynthetic events involved in maltase induction by measurement of the following: (i) the change in maltase-specific activity, (ii) the in vivo rate of maltase synthesis, (iii) the accumulation of maltase functional mRNA, (iv) the accumulation of RNA sequences detected by filter hybridization to the cloned structural gene, and (v) the in vivo rate of maltase structural gene transcription.

MATERIALS AND METHODS

Yeast. *S. carlsbergensis* strain CB11 (**a** *mal1*p *MAL1g mal3p MAL3g MAL6p MAL6g ade1*) was obtained from A. M. A. ten Berge. This strain was selected for study because of the extensive investigations by ten Berge and his associates (21) on maltase synthesis.

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The chemicals used in this study were maltose (Difco Laboratories), dextrose (Sigma Chemical Co.), agarose (Sigma; low EEO), [^{35}S]methionine (Amersham Corp.; 600 Ci/mmol), [^{3}H]uracil (Schwarz/Mann; 20 Ci/mmol), [^{32}P]deoxycytidine 5′-triphosphate (Amersham; 2,000 Ci/mol), [^{32}P]adenosine 5′-triphosphate (5,000 Ci/mmol), poly-*U*-Sepharose (Pharmacia), and *Staphylococcus aureus* protein A Sepharose (Pharmacia). All other chemicals were reagent grade. Plasmid p*tcm*(3.2), carrying the yeast ribosomal gene L3 (encoding trichodermin resistance), was a gift from H. Fried and has been described previously (6). Plasmid pMAL PE8 is a subclone of the maltase structural gene (5) constructed by one of the authors (H.J.F.). It contains a *Pst*I to *Eco*RI restriction fragment, representing an internal portion of the maltase structural gene transcription unit, cloned into pBR325 (H. J. Federoff, unpublished result).

Growth of cells. A stock culture of strain CB11 was maintained on YPD agar slants containing 1% yeast extract (Difco), 2% peptone (Difco), and 2% dextrose. Cells were first grown in SC medium (1% succinic acid, 0.6% sodium hydroxide, 0.67% yeast nitrogen base [Difco]) plus 5% dextrose, supplemented with 50 μg of adenine (sulfate or free base) per ml, to midlogarithmic phase at 30°C. The cells were harvested by centrifugation and then suspended in induction medium (SC medium plus 2% maltose and 0.1% dextrose, supplemented with 50 μg of adenine per ml).

Enzyme assay. Maltase activity was assayed in a coupled assayed system. The assay mixture contained PM buffer (50 mM potassium phosphate, 1 mM β-mercaptoethanol), 4 mM $MgCl_2$, 0.45 mg of NADP per ml, 10 mM ATP, 2 mM maltose, 1.4 U of hexokinase (140 U/mg), and 0.35 U of glucose-6-phosphate dehydrogenase (350 U/mg) (both enzymes obtained from Boehringer Mannheim Corp.). The reduction of NADP to NADPH was measured at 340 nm by using a Gilson recording spectrophotometer after the addition of a disrupted yeast cell extract dialyzed overnight against PM buffer.

RNA isolation, in vitro RNA labeling, and filter

hybridizations. The isolation of total and polyadenylated [poly(A)$^+$]RNA was performed by the method of Federoff et al. (5). Fragmented poly(A)$^+$ RNA was labeled at 5′ ends with polynucleotide kinase and γ-[^{32}P]ATP by the procedure described by Chaconas and van de Sande (1). The poly(A)$^+$ RNA was subjected to mild alkaline hydrolysis (30 mM Tris-sulfate [pH 9.0] for 15 min at 80°C) before end labeling. Filter hybridizations were performed by the method of Kim and Warner (9), except that the filters were loaded with 15 μg of denatured plasmid DNA and prewashed in hybridization solution before incubation with radioactive RNA. The hybridization solution contained 20 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.8).

RNA gel transfers. Total yeast RNA was denatured, reacted with formaldehyde, electrophoresed through a 1.5% agarose gel containing formaldehyde, and blotted to nitrocellulose as previously described (16, 18). Radiolabeling of DNA by nick translation was performed by the method of Rigby et al. (17).

In vivo labeling of cells, in vitro translation, and immunoprecipitation. For protein labeling, 2 ml of yeast cells (density, 1×10^7 to 3×10^7 cells per ml) were incubated with 400 μCi of [^{35}S]methionine per ml for 5 min and chased for 1 min with a 1,000-fold molar excess of unlabeled methionine to allow completion of nascent polypeptide chains. After labeling, cells were washed with sterile water and frozen. For the measurement of maltase synthesis, cells were thawed, suspended in 400 μl of buffer containing phosphate-buffered saline (10 mM sodium phosphate [pH 7.0], 150 mM NaCl), 1% Triton X-100, and 5 mM phenylmethylsulfonyl fluoride. Glass beads were added, and the cells were disrupted in a Braun homogenizer with cooling. After the extent of incorporation of ^{35}S into protein (specific activities varied from 0.5×10^6 to 3.5×10^6 cpm/μg) was determined, extracts were immunoprecipitated. For RNA labeling, yeast cells were labeled with [^{3}H]uracil at a final concentration of 200 μCi/ml of cells, washed with sterile water, frozen, and treated as described above for RNA isolation.

The preparation of the wheat germ extract for the cell-free translation system and translation of poly(A)$^+$ RNA was performed by the method of Marcu and Dudock (11). The amount of poly(A)$^+$ RNA input was varied, and the yield of radioactivity incorporated into trichloroacetic acid-insoluble material was found to show a linear response between [^{35}S]methionine incorporation into protein and RNA input.

Antibodies were raised in rabbits by injecting purified maltase in Freund complete adjuvant and boosting in incomplete Freunds adjuvant until antibody titers rose. Sera collected at weekly intervals were titrated by standard procedures (8). The total immunoglobulin fraction was prepared from antisera with maximum titers (8). Antibody titration curves were performed with respect to ^{35}S in vivo- or in vitro-labeled protein for each fresh antibody preparation to establish conditions of antibody excess. Quantitative immunoprecipitation was accomplished as follows. To a sample of in vivo- or in vitro-labeled extract containing 10^7 cpm brought to a final volume of 100 μl was added 100 μl of anti-maltase gamma globulins (protein concentration, 0.5 mg/ml) in phosphate-buffered saline containing 1% Triton X-100. Immune complex formation proceeded overnight at 4°C. Immunoglobulins were complexed by the addition of a volume of *S. aureus* protein A Sepharose (20% [wt/vol]) slurry sufficient to complex all gamma globulins (determined by titrating ^{125}I tracer-labeled gamma globulins with protein A Sepharose) for 2 h at 4°C with rotation. After extensive washing of the beads to minimize nonspecific trapping, immune complexes were dissociated in sample loading buffer containing sodium dodecyl sulfate (2%) and dithiothreitol (5 mM), loaded on a 12.5% polyacrylamide gel, and electrophoresed by the method of Douglas and Butow (4). Dried gels were autoradiographed, the appropriate bands were excised and solubilized in 30% hydrogen peroxide at 80°C for 5 h, and their radioactivity was determined in a liquid scintillation counter.

RESULTS

Prior investigations of maltase induction involved quantitation of enzyme activity as a function of changes in growth conditions (23–26). To begin to understand the molecular events leading to the appearance and accumulation of maltase, the following parameters were investigated as a function of time during induction: (i) the change in maltase specific activity, (ii) the in vivo rate of maltase synthesis, (iii) the accumulation of functional maltase mRNA, (iv) the accumulation of hybridizable maltase RNA sequences, and (v) the in vivo rate of maltase gene transcription

The measurement of maltase activity was performed by a coupled assay with maltose as a substrate. The following protocol for maltase induction was used for all measurements. Cells of strain CB11 were pregrown overnight under conditions in which the accumulation of maltase activity was repressed, i.e., SC medium plus 5% dextrose, supplemented with adenine. Cells were harvested, washed with SC medium, and suspended in warmed (30°C) induction medium (SC plus 2% maltose and 0.1% dextrose, supplemented with adenine). Maltase specific activity (Fig. 1) was determined from cells removed from the culture at the times indicated after inducer addition. The data reveal a lag period of approximately 2.5 h before the maximal rate of maltase accumulation was achieved. The lag has been termed adaptation (3), reflecting the time required to relieve glucose repression and to initiate the sequence of events leading to maltase appearance.

To determine whether induced maltase accumulation is due to de novo enzyme synthesis rather than the conversion of preexisting inactive to active enzyme, the rate of maltase synthesis was examined. Cells were induced as described above: samples containing an equal number of cells were removed, pulse-labeled with [^{35}S]methionine for 5 min, chased for 1 min to allow completion of nascent proteins chains, and quantitatively immunoprecipitated. Anti-

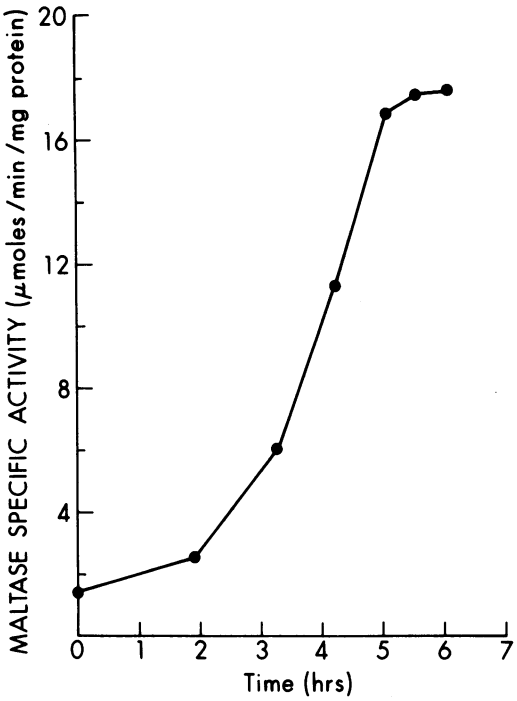

FIG. 1. Kinetics of maltase accumulation. Cells of strain CB11 grown for 14 h in SC medium plus 5% dextrose supplemented with 50 μg of adenine sulfate per ml were harvested, washed, and suspended in induction medium. At the times indicated, samples of the cell culture (each containing an equal number of cells) were removed, frozen, and subsequently assayed for maltase activity by using the coupled assay (see text).

sera were titrated with respect to input radioactivity to ensure that precipitations were performed in antibody excess. Titration curves similar to the one shown in Fig. 3C were performed. Immunoprecipitates were analyzed by electrophoresis on a 12.5% sodium dodecyl sulfate-polyacrylamide gel and autoradiographed to determine the location of the maltase band. Gel bands were excised and solubilized in hydrogen peroxide, and their radioactivity was quantitated. The results (Fig. 2) indicate that (i) no significant maltase synthesis occurs at time zero nor is any labeled, antigenically related precursor made upon induction; (ii) approximately 3 h is required before a significant rate of synthesis is achieved; and (iii) a decline in the synthetic rate is seen after the maximal rate is achieved. The results suggest that de novo synthesis of maltase leads to enzyme appearance and accumulation.

The role of de novo RNA transcription was examined in a series of three experiments. The first experiment measured the amount of functional maltase mRNA assayed by in vitro translation of poly(A)$^+$ RNA in a wheat germ-derived system. The quantitation of functional maltase mRNA requires (i) the assumption that the amount of maltase synthesized in vitro as assayed immunologically is proportional to the amount of maltase mRNA and (ii) that controls be performed for each RNA sample to demonstrate that there is a linear response between poly(A)$^+$ RNA input into the cell-free translation system and the amount of radioactivity

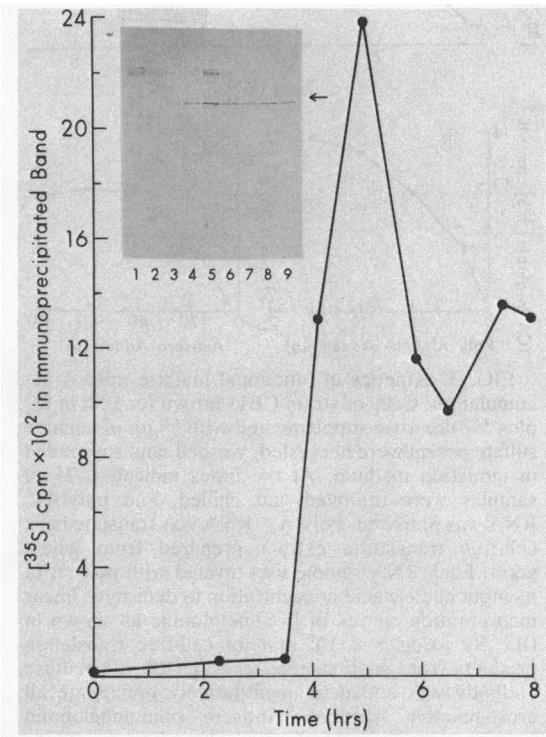

FIG. 2. Kinetics of rate of maltase synthesis. Cells of strain CB11 grown for 8 h in SC medium plus 5% dextrose supplemented with 50 μg of adenine base per ml were harvested, washed, and suspended in induction medium. At the times indicated, samples of the cell culture (each containing an equal number of cells) were removed, pulse-labeled for 5 min with 400 μCi of [^{35}S]methionine per ml, chased for 1 min with a 1,000-fold molar excess of unlabeled methionine, and frozen. Cells were thawed and disrupted, protein specific activity was determined, and equal input counts per minute were quantitatively immunoprecipitated. Antibody titration curves were performed to determine conditions of antibody excess (Fig. 3C). Immunoprecipitates were analyzed on a 12.5% sodium dodecyl sulfate-polyacrylamide gel, and bands were localized by autoradiography (inset), excised, and counted. The arrow indicates the migration position of purified maltase. Data are expressed as counts per minute per excised band.

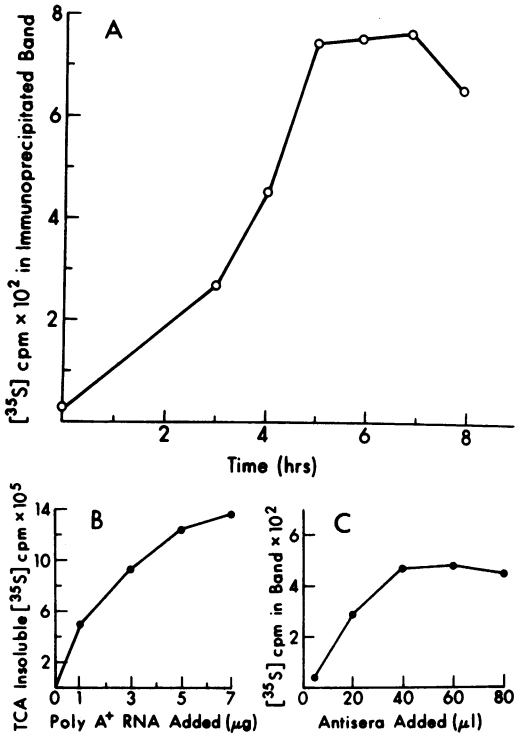

FIG. 3. Kinetics of functional maltase mRNA accumulation. Cells of strain CB11 grown for 12 h in SC plus 5% dextrose supplemented with 50 μg of adenine sulfate per ml were harvested, washed, and suspended in induction medium. At the times indicated, 25-ml samples were removed and chilled, and poly(A)$^+$ RNA was prepared. Poly(A)$^+$ RNA was translated in a cell-free translation extract prepared from wheat germ. Each RNA sample was titrated with respect to its input nucleic acid concentration to determine linear incorporation curves of [^{35}S]methionine as shown in (B). By using 5 × 10^6 cpm of cell-free translation products from each sample, excess (100 μl) maltase antibody was added to quantitatively precipitate all cross-reactive material. Antisera (immunoglobulin fraction) titration curves were plotted to determine conditions of antibody excess (C). Protein A Sepharose was added to complex all immunoglobulins. The immunoprecipitates were analyzed on a 12.5% polyacrylamide gel, and the bands were located by autoradiography, excised from the gel, and counted in a scintillation counter. The data are expressed as counts per minute of ^{35}S determined in each excised band. TCA, Trichloroacetic acid.

incorporated into protein. An induction experiment was performed in which samples of the culture were removed periodically for total poly(A)$^+$ RNA preparation. RNA translation titrations were done for each RNA sample to determine the linearity of the response. An example of one such titration is shown in Fig. 3B. The poly(A)$^+$ RNA-programmed cell-free translation products were quantitatively immunoprecipitated, each was analyzed on a gel, bands were identified and excised, and radioactivity was then determined. The radioactivity in each band represents the amount of ^{35}S incorporated into maltase as directed by poly(A)$^+$ RNA isolated from cells during the induction. The data demonstrate that functional maltase mRNA does not exist at time zero, that functional mRNA is inducible, and that, after reaching its peak accumulation value, the effective amount of functional mRNA declines.

To exclude the possibility that nonfunctional precursor maltase RNA is made constitutively and processed to functional mRNA after inducer addition, total maltase RNA sequence accumulation was examined. Poly(A)$^+$ RNA was prepared from culture samples taken at various times after induction. The RNA was fragmented by treatment with mild alkali, 5′ end labeled with γ-[^{32}P]ATP and polynucleotide kinase, and then hybridized to excess denatured plasmid DNA immobilized on nitrocellulose filters. The plasmid pMAL PE8 contains a restriction fragment internal to the maltase structural gene transcriptional unit. Specific hybridized radioactivity, expressed as the percentage of input radioactivity, was plotted as a function of time during induction. The data (Fig. 4) indicate that essen-

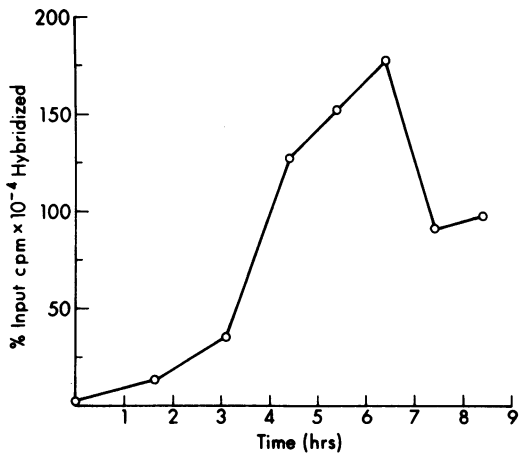

FIG. 4. Kinetics of accumulation of maltase mRNA sequences. Cells of strain CB11 grown for 12 h in SC medium plus 5% dextrose supplemented with 50 μg of adenine sulfate per ml were harvested, washed, and suspended in induction medium. At the times indicated, 10-ml samples of the cell culture were chilled, and poly(A)$^+$ RNA was prepared. The poly(A)$^+$ RNA was fragmented by treatment with mild alkali, 5′ end labeled with γ-[^{32}P]ATP and polynucleotide kinase, and hybridized to nitrocellulose filters containing denatured immobilized pMAL PE8 DNA. Data represent the average of duplicate filter hybridizable counts after subtraction of background and are expressed as percentage of input counts per minute hybridized.

tially no RNA complementary to a segment of the maltase structural gene was detected at time zero. Appreciable maltase RNA sequence accumulation was seen at 3 h and achieved a maximal level at 6.5 h. A decline in the amount of maltase RNA sequence was seen after 6.5 h, paralleling the decline in the rate of maltase enzyme synthesis seen previously.

Additional evidence that this de novo RNA accumulation represents mature maltase mRNA is provided by RNA Northern blot analysis. Equal amounts of total RNA, prepared from culture samples removed at various times during induction, were denatured, reacted with formaldehyde, electrophoresed through a 1.5% agarose gel, blotted to nitrocellulose, and probed with radiolabeled pMAL PE8 DNA (Fig. 5A). The size of the mature maltase mRNA was previously determined to be approximately 1,900 nucleotides (H. J. Federoff, unpublished observation). Examination of Fig. 5A reveals that no mature maltase mRNA sequences or other molecular weight species hybridizable to the maltase structural gene probe were detected at time zero, i.e., at the time of inducer addition (Fig. 5A, lane 1). Progressively increasing accumulation of mature maltase mRNA was seen as a function of increasing time during induction. In a control experiment, hybridization of the same blot to radiolabeled p*tcm*(3.2), a plasmid containing the gene for ribosomal protein L3 (6) (Fig. 5B), showed that the changes seen in the levels of maltase mRNA were not a function of RNA loading and that the amounts of another mRNA remained relatively constant during maltase induction. The data from the peceding three experiments, taken together, demonstrate that the appearance and accumulation of maltase is a consequence of the de novo appearance of mature, functional maltase mRNA.

The last parameter examined was the rate of maltase gene transcription during induction. Samples of a culture removed at various times during an induction experiment were pulse-labeled for 8 min with [^{3}H]uracil. The length of the pulse time represents between 30 and 40% of the half-life determined for the maltase mRNA (10; H. J. Federoff and T. R. Eccleshall, unpublished results). Total RNA was prepared from each sample and hybridized to plasmid pMAL PE8 immobilized on nitrocellulose filters, and the amount of specific hybridization was determined. The data (Fig. 6) indicate that at time zero very low transcriptional rates were measurable and that the maximal rate of transcription was achieved 90 min after inducer addition. There was approximately a 15-fold increase in the rate of transcription at 90 min relative to time zero. As a control, the rate of transcription of a gene encoding the ribosomal protein L3 is shown. The rate of transcription of this gene was seen to decrease approximately twofold during adaptation of cells previously grown on dextrose as a carbon source upon transfer to maltose-containing medium. This decrease probably reflects in part the difference in the cellular growth rate in the two different media. This effect has been observed for other ribosomal protein genes (J. Warner, personal communication).

DISCUSSION

S. carlsbergensis synthesizes a maltose inducible and glucose-repressible α-glucosidase or maltase. The enzyme is a monomer of 63,000 daltons (14). In response to a change in the carbon source in the growth medium from glucose to maltose, there is a coordinate induction

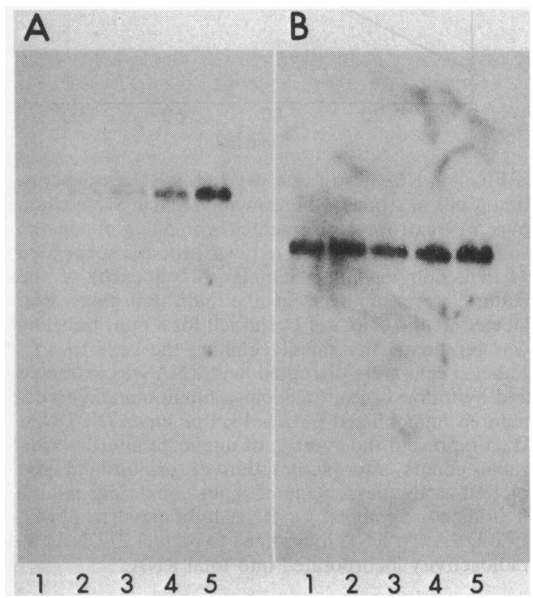

FIG. 5. Identification of mature maltase mRNA during induction. Cells of strain CB11 grown for 10 h in SC medium plus 5% dextrose supplemented with 50 μg of adenine sulfate per ml were harvested, washed, and suspended in induction medium. As a function of time after induction, 50-ml samples of the cell culture were removed and frozen. A 5-μg amount of total RNA prepared from each sample was denatured, reacted with formaldehyde, and electrophoresed through a 1.5% agarose gel containing formaldehyde. The gel was blotted to nitrocellulose, baked, and hybridized with plasmid DNAs radiolabeled by nick translation. (A) Blot probed with pMAL PE8 DNA. Lanes represent RNA prepared from cells taken at various times after suspension in induction medium. Lane 1, time zero; lane 2, 1 h; lane 3, 2 h; lane 4, 3.5 h; lane 5, 5 h. (B) Same blot probed with ribosomal protein gene containing plasmid p*tcm*(3.2) DNA. Lanes are the same as those in (A).

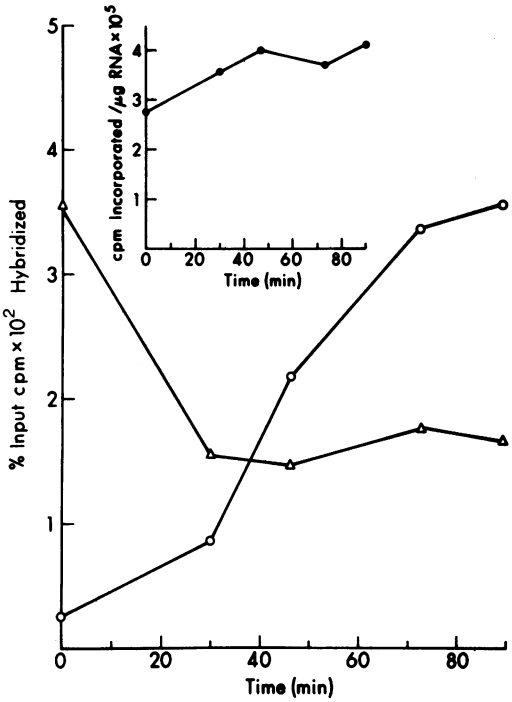

FIG. 6. Kinetics of rate of *MAL* mRNA transcription. Cells of strain CB11 grown for 12 h in SC medium plus 5% dextrose supplemented with 50 μg of adenine sulfate per ml were harvested, washed, and suspended in induction medium. Samples (2 ml each) of the culture were removed at the indicated times and labeled with 400 μCi of [^{3}H]uracil for 8 min. Labeling was terminated by rapidly chilling the cells to 4°C. Labeled cells were disrupted, and RNA was extracted and hybridized to nitrocellulose filters containing denatured immobilized pMAL PE8 or p*tcm*(3.2) DNA. Data represent the average of duplicate filter hybridizable counts after subtraction of background and plotted as the percentage of input counts per minute hybridized. Symbols: ○, RNA hybridized to pMAL PE8 DNA; △, RNA hybridized to p*tcm*(3.2) DNA; ●, radioactivity incorporated into total RNA.

of maltase and a maltose-specific transport system (2).

Prior investigations on the control of maltase induction involved the measurement of changes in enzyme activity in response to changes in growth conditions (23, 24, 26) or the addition of inhibitors of RNA and/or protein synthesis (25). Interpretation of these earlier data is difficult for several reasons. (i) Measurement of total enzyme activity is not a sensitive index of changes occurring transcriptionally, post-transcriptionally, or at the translational level. (ii) The substrate used for most of these studies, *p*-nitrophenyl-α-D-glucoside, is also hydrolyzed by the maltose-inducible enzyme α-methyl glucosidase (15). The latter enzyme is a physically distinct molecule encoded by a different family of structural genes. This investigation reexamines the mechanisms of maltase induction utilizing direct methods.

The time course of maltase activity accumulation is characterized by a lag, termed adaptation, of approximately 2.5 h before an appreciable rate of enzyme accumulation is observed. The appearance and accumulation of maltase activity could be due to de novo enzyme synthesis or simply the conversion of inactive to active enzyme. It had been previously demonstrated (25) that *p*-nitrophenyl-α-D-glucosidase appearance and accumulation in spheroplasts were inhibited by the addition of the protein synthesis inhibitor cycloheximide. This result suggested that de novo protein synthesis was required for the appearance of active enzyme. The experiments presented here, employing methods that measure maltase synthesis directly, confirm that de novo synthesis of maltase is obligately coupled to enzyme appearance and accumulation.

The requirement for maltase structural gene transcription was addressed in a series of four experiments. First, it was shown that induction involves the de novo appearance of functional maltase mRNA. Second, the examination of total RNA sequences complementary to a maltase structural gene probe revealed that these sequences appear and accumulate de novo. Third, the appearance and accumulation of mature maltase mRNA were shown to occur as a function of induction. Last, the direct demonstration that maltase gene transcription is stimulated as a function of inducer addition was presented. Taken together, these data indicate that stimulation of transcription of the maltase structural gene occurs during the initial phase of induction, preceding the accumulation of mature functional maltase mRNA and its translation product, maltase. These data are in agreement and extend the previous finding of inhibition of *p*-nitrophenyl-α-D-glucosidase appearance and accumulation by actinomycin D added during the initial phase of induction (25).

Examination of the time course of the change in the maltase synthetic rate and total hybridizable maltase RNA sequences reveals an interesting feature of induction. After reaching a maximal value, either in the rate of maltase synthesis or maltase mRNA accumulation, a rapid decline of both parameters followed.

The data presented here, along with those available in the literature (2, 22–26), suggest a model for the sequence of events leading to the induction of maltase. It is proposed that cells growing in dextrose have a very low rate of maltase structural gene transcription. When dextrose is replaced by maltose in the growth medium, the disaccharide first enters the cell via

a facilitated process (7). Upon entry into the cell, maltose interacts with a regulatory gene product(s), leading to an approximately 15-fold increase in the rate of maltase gene transcription. Presumably, the transcription of the gene encoding the maltose permease is similarly stimulated. Translation of these mRNAs leads to the active transport of maltose and its concomitant hydrolysis by maltase. Once induction is well under way, the intracellular glucose concentration rises as a consequence of maltose hydrolysis and exerts a repressive effect (H. J. Federoff and T. R. Eccleshall, unpublished results) on the maltase synthetic capacity of the cell.

It should be pointed out that the genes involved in maltose metabolism in yeasts constitute a multigene family of five unlinked loci (*MAL1-MAL4* and *MAL6*). From genetic (12, 13) and biochemical (Southern blot) analysis (M. Goldenthal, T. Chow, J. Cohen, and J. Marmur, unpublished results), it appears that each *MAL* locus is comprised of at least two linked genes: a regulatory gene, *MALp*, and a maltase structural gene, *MALg*. The analysis of the strain used in this study (CB11) has revealed that it contains three functional maltase structural genes (*MAL1g*, *MAL3g*, and *MAL6g*) and a single functional regulatory gene (*MAL6p*). It is not clear that the study presented here (with CB11) represents measurement of the expression of one or all of these maltase structural genes.

The isolation and characterization of the galactose gene cluster from *S. cerevisiae* (19) has provided another system for the study of regulation of inducible genes. Detailed transcriptional analysis of this gene cluster suggests that the promoters for each of the three genes are coordinately regulated (20). Similar studies on a complete *MAL* locus are in progress.

In summary, this report establishes that maltase induction involves a significant increase in the rate of transcription with concomitant functional mRNA appearance, followed by de novo enzyme synthesis, resulting in the appearance and accumulation of enzyme activity.

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