mRNA Levels for the Fermentative Alcohol Dehydrogenase of *Saccharomyces cerevisiae* Decrease upon Growth on a Nonfermentable Carbon Source*

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The classical, fermentative alcohol dehydrogenase from *Saccharomyces cerevisiae*, which previously was thought to be constitutive, has been shown to be repressed by growth on nonfermentable carbon sources.

The rate of alcohol dehydrogenase I protein synthesis declined 6-fold within 3 to 4 h after yeast were transferred from medium containing glucose to medium containing ethanol, and it declined 10-fold after glucose became depleted from the medium during diauxic growth. The decreased rate of alcohol dehydrogenase I protein synthesis was shown not to be the result of an increased rate of degradation of the alcohol dehydrogenase I protein.

The decline in alcohol dehydrogenase I protein synthesis was correlated with a 6- to 10-fold decrease in the amount of functional alcohol dehydrogenase I mRNA within 3 to 4 h after transfer from glucose-containing medium to medium containing ethanol. A similar decrease in alcohol dehydrogenase I functional mRNA occurred when cells were depleted of glucose by diauxic growth. Total alcohol dehydrogenase I mRNA, as detected by hybridization to the cloned ADCl gene, was found in the same relative abundance as the amount of translatable alcohol dehydrogenase I mRNA during the different growth conditions. These results suggest that the alcohol dehydrogenase I protein is transcriptionally regulated.

When yeast are grown on a fermentable carbon source such as glucose, the fermentative, classical alcohol dehydrogenase (ADH I) catalyzes the regeneration of NAD" from NADH with the concomitant production of ethanol from acetaldehyde. During such growth ADH I is present in a large amount in the cell as are many of the other principle fermentative enzymes in yeast (1, 2). However, when the fermentable carbon source is depleted, the yeast cells derepress the synthesis of a variety of other enzymes in order to utilize the previously excreted ethanol as an energy and carbon source via oxidative respiration and gluconeogenesis. Instead of using ADH I for the reverse reaction in the utilization of ethanol, another ADH isozyme, the glucose-repressible ADH (ADH II) is synthesized (3). This latter enzyme seems kinetically better suited for the reverse reaction than ADH I (4).

Previous work on the control of yeast ADHs assumed on the basis of ADH I enzyme activity that it was synthesized constitutively (3, 5), i.e. synthesized in fixed amounts irrespective of the carbon source (10). However, there is no obvious need for yeast to maintain the synthesis of two ADHs during growth on ethanol. It seemed reasonable to investigate whether ADH I was indeed synthesized at comparable levels during growth on fermentable and nonfermentable carbon sources. In addition, it also seemed important to study the expression of ADH I since its promoter is being used to obtain the synthesis of foreign proteins in yeast (25-27). The results presented in this paper indicate that the amount of ADH I mRNA and the rate of ADH I protein synthesis decrease upon growth in ethanol-containing medium and during growth into stationary phase. The inverse regulation of the ADH isozymes and its relationship to the other fermentative enzymes in yeast is discussed.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

**ADH I Protein Synthesis**—The rate of ADH I protein synthesis during growth on a nonfermentable carbon source was quantitated by a radioimmune assay. Yeast were grown on glucose-containing medium overnight and transferred to medium containing ethanol. At various times after transfer, the yeast cells were pulse-labeled for 15 min with ["S]methionine, and the ADH protein was isolated by immunoprecipitation with antibody specific to ADH (1). The total radioactive proteins and the radioactive immunoprecipitates were subsequently fractionated by SDS-polyacrylamide gel electrophoresis and identified by fluorography. Densitometric analysis of these fluorograms was carried out in order to calculate the percentage of total radioactively labeled protein which was ADH (6). Because the strains used in these experiments contained both ADH I and ADH II activity, ADH I protein had to be differentiated from that of its related and anti-

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§ The abbreviations used are: ADH 1, alcohol dehydrogenase (EC 1.1.1.1); SDS, sodium dodecyl sulfate.

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* Portions of this paper (including "Experimental Procedures" and Figs. 3, 4, and 6-8) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-1661, cite authors, and include a check or money order for $5.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
cally similar isozyme ADH II-F. In the first two series of experiments, ADH I and ADH II-F were distinguished after limited proteolysis of the immunoprecipitates with Staphylococcus aureus protease (6, 9). This method was used previously to quantitate ADH II-F protein synthesis (6). Fig. 1 displays a typical fluorogram of the total proteins synthesized after transfer of yeast to medium containing ethanol, and Fig. 2 displays the proteolytic cleavage patterns of the immunoprecipitated ADH.

As can be seen by the distribution of radioactive polypeptides in Fig. 1, significant changes in the synthesis of some polypeptides occurred when yeast were transferred to medium containing ethanol, although the total rate of [35S]methionine incorporation remained relatively unchanged. The data in Fig. 2 indicate that ADH I protein synthesis declined in the first 2 h of growth on a nonfermentable carbon source. Synthesis of ADH II was first detected approximately 1 h after release from glucose repression. This result is in substantial agreement with previous results on ADH II regulation, which was analyzed in strains deficient in ADH I enzyme activity (6).

Fig. 3a (see Miniprint) depicts the change in the percentage of both ADH I and ADH II protein synthesis during growth on ethanol-containing medium. The values presented are the average of three separate experiments. The proportion of ADH I protein synthesis decreased from approximately 6% of total protein synthesis in the presence of glucose (first time point) to about 1% of total protein synthesis 3 to 4 h after transferring the cells to medium containing ethanol. ADH I protein synthesis after this time remained constant or increased slightly. The results for ADH II protein synthesis are in agreement, as mentioned above, with previous data which were obtained using strains lacking ADH I activity (6).

**ADH I Protein Turnover**—The rate of ADH I protein degradation was measured to determine if the apparent decline in ADH I protein synthesis resulted from an increased turnover of ADH I protein during growth on ethanol. Yeast grown in medium containing either glucose or ethanol were pulse-labeled with [35S]methionine for 15 min, washed, and chased with nonradioactive methionine. At times up to 2 h after the chase, samples were taken and ADH I protein synthesis was quantitated as described above. In addition, a fraction of the original yeast culture was grown continuously on glucose in the presence of [35S]methionine in order to show that incorporation of [35S]methionine into protein would have proceeded unimpeded throughout this interval. Fig. 4 (see Miniprint) depicts the change in total [35S]methionine incorporation and ADH I synthesis during the experiment.

In cells continuously incubated with [35S]methionine, radioactivity accumulated linearly with respect to time into total protein (Fig. 4a, Miniprint) and into ADH I (Fig. 4b, Miniprint). In cells labeled with [35S]methionine and then grown on either glucose- or ethanol-containing media in the presence of excess nonradioactive methionine, total incorporation of radioactivity into proteins did not change after removal of the radioactive label (Fig. 4a). This control experiment indicates that the chase with unlabeled methionine had stopped the incorporation of exogenous radioactivity into yeast proteins. Fig. 4b depicts the change in the amount of radioactivity in the immunoprecipitated ADH I protein when cells were labeled with [35S]methionine and then incubated in the presence of nonradioactive methionine. In cells growing on medium containing ethanol, no turnover of ADH I protein was observed while in cells growing in medium containing glucose, a slight increase in radioactive ADH I protein was observed. These results strongly suggest that the decline in ADH I protein synthesis during growth on a nonfermentable carbon source was not a result of a major change in the rate of ADH I protein degradation upon switching cells to medium containing ethanol, nor was it a result of a high turnover rate for the ADH I protein.

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1 ADH II-F refers to the fast migrating allele of ADH II as differentiated from ADH II-S (slow) when the native proteins are subjected to starch gel electrophoresis (30).

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**Fig. 1.** Fluorogram of 10% SDS-polyacrylamide gel containing radioactive yeast polypeptides. Proteins were pulse-labeled for 15 min with 20 μCi/ml of [35S]methionine. Forty μl of each sample were loaded into each slot. The lanes represent the times in hours when pulse-labeling with [35S]methionine occurred after the cells from strain 79-72C were transferred from glucose-containing medium to medium containing ethanol. The molecular weight markers were catalase (58,000), ovalbumin (43,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), and carbonic anhydrase (29,000).

**Fig. 2.** A 15% SDS-polyacrylamide gel of S. aureus protease cleavage patterns of ADH. Limited proteolysis, electrophoretic conditions, protein staining, and fluorography have been described previously (6). a, nonradioactive ADH I purified from strain 300-9: MATα ade2 trp2 ADC1 ade2-2 ads; b, nonradioactive ADH II-F purified from strain 11-13C: MATα trp2 ade1-11 ADR2-S ads. The remaining lanes are fluorograms of immunoprecipitated ADH from strain 79-72C which was radioactively labeled during growth on ethanol-containing medium. The values in hours represent the times when the pulse-labeling with [35S]methionine occurred after the cells were transferred to ethanol-containing medium. The molecular weights designate the sizes of the principal ADH proteolytic cleavage fragments (6).
Amount of Translatable ADH I mRNA—To determine whether the decline in ADH I protein synthesis resulted from a change in the amount of ADH I mRNA, the amount of translatable ADH I mRNA was measured. RNA was extracted from yeast at various times after placing them in medium containing ethanol. The RNA was translated in a wheat germ cell-free system in the presence of \(^{14}C\)methionine after which the radioactive ADH was immunoprecipitated from a portion of the sample. The immunoprecipitate and a sample of the total radioactive proteins were analyzed by polyacrylamide gel electrophoresis and fluorography. The percentage of total protein synthesis which was ADH I was calculated as described in the preceding sections after analyzing the immunoprecipitated ADH by limited proteolysis. Fig. 3b (see Miniprint) depicts the change in the percentage of ADH I and ADH II synthesis \textit{in vitro} (equivalent to the amount of translatable ADH I and ADH II mRNA) as a function of time after transfer of the yeast to ethanol-containing medium. The values represent the average of three separate experiments.

As seen in Fig. 3b (see Miniprint), the percentage of functional ADH I mRNA declined from approximately 1.1 to 0.2% within 3 to 4 h after switching the cells to ethanol-containing medium. This decline was coincident with the change in ADH I protein synthesis as seen in Fig. 3a. After 3 h, the amount of translatable ADH I mRNA leveled off. The amount of translatable ADH I mRNA and the timing of its appearance were similar to that previously found in cells deficient in ADH I activity, \textit{i.e.} ADH II mRNA appeared after about 1 h (6).

These results were confirmed using a more direct assay for \textit{in vitro}-synthesized ADH I and ADH II protein by separating by size the ADH I and ADH II-S proteins. On SDS-containing polyacrylamide gels, the subunit polypeptides of ADH II-S migrate with a lower apparent molecular weight than the subunits of ADH I. The separation of ADH II-S and ADH I polypeptides on an SDS-polyacrylamide gel is shown in Fig. 5, a-c. A third band was sometimes observed which migrated beneath ADH II-S (see lane c). This third band was considered to be a derivative of ADH I since it was only present when ADH I was present. In the original photographs, this ADH I band was present at the first two time points described below.

The amount of functional ADH I and ADH II-S mRNA present during growth on glucose-containing medium and during growth on ethanol-containing medium was determined in an experiment similar to the one described above. Total RNA was extracted at various times after transfer of the cells at time zero from medium containing ethanol to that containing glucose. The reciprocal experiment was also performed: RNA was extracted at various times after transfer of cells at time zero from glucose-containing medium to that containing ethanol. Equal microgram quantities of RNA were translated \textit{in vitro} in the presence of \(^{14}C\)methionine. The radioactive ADH polypeptides were immunoprecipitated and identified by fluorography after separation on SDS-polyacrylamide gels.

A fluorogram showing the change in the relative levels of ADH I and ADH II-S mRNA as a function of time is presented in Fig. 5, and this change is depicted quantitatively in Fig. 6 (see Miniprint). In Fig. 5, a polypeptide of unknown origin can be seen to have precipitated along with ADH. This species was not observed in the immunoprecipitates in the previous experiments, suggesting that it was not an ADH degradation product produced in the cell-free translation system. Transfer from ethanol growth medium to medium containing glucose resulted in about a 2- to 5-fold increase in ADH I mRNA level with a corresponding 4-fold decrease for the amount of ADH II mRNA (Fig. 6a, Miniprint). In the reciprocal experiment, transfer from medium containing glucose to medium containing ethanol (Figs. 5 and 6b), ADH I mRNA declined 10-fold within 3 to 4 h. At the same time, ADH II mRNA became derepressed. These results are similar to those obtained above using limited proteolysis to distinguish the two enzymes, although the degree of ADH I mRNA decline appears to differ in the two experiments. However, this difference is probably attributable to the fact that the data obtained by separating the ADH isozymes by size were not normalized to the total amount of translatable mRNA but to the amount of RNA present as measured by its absorbance at 260 nm. These combined results indicate that the decrease in ADH I protein synthesis upon growth on a nonfermentable carbon source resulted from a decrease in the amount of translatable ADH I mRNA.

**ADH I Regulation during Growth into Stationary Phase—** ADH I protein synthesis and mRNA levels were also analyzed in cells inoculated into medium containing glucose and grown continuously into stationary phase. These experiments were undertaken in order to analyze ADH regulation in conditions in which the glucose became depleted naturally from the medium. Cells from a stationary phase culture of strain 358-21 were inoculated into medium containing 1% glucose and allowed to grow for 26 h. Between 14 and 16 h after inoculation, the glucose was depleted from the medium and cell division ceased (data not shown). A second period of cellular division, albeit at a much slower rate than the initial logarithmic growth, commenced at about 20 h (22). The rate of ADH protein synthesis was measured as described above.

Fig. 7a (see Miniprint) depicts the rate of ADH I and ADH II protein synthesis as function of time during the course of the experiment. The lack of ADH protein synthesis until 4 h reflected the fact that little total protein synthesis occurred until that time. This resulted from the use of an inoculum that was taken from stationary phase cells that were essentially dormant. ADH I was synthesized during the growth on glucose medium, but its rate of synthesis declined about 5-fold between 12 and 14 h after the start of the experiment. This
decline in ADH I protein synthesis was coincident with the cessation of cellular division and the depletion of glucose from the medium (22). Little or no ADH II protein synthesis occurred when glucose was present in the medium, but ADH II synthesis increased after the depletion of glucose from the medium, which is in agreement with previous results on the study of ADH II regulation (see above) (6).

The amount of functional ADH I mRNA was quantitated as described above by extracting RNA at times throughout the growth into stationary phase, translating the RNA in the cell-free system, and identifying the amount of ADH I poly-peptide synthesized by limited proteolysis and fluorography. Fig. 7b (see Miniprint) presents the percentage of total translatable mRNA which was respectively ADH I and ADH II during growth into stationary phase. The amount of functional ADH I mRNA was found to decline at the same time that ADH I protein synthesis was shown to decline in Fig. 7a. These results indicate that the decline of ADH I functional mRNA levels was observed when cells were transferred to medium containing ethanol also occurred when cells were depleted of glucose by growth into stationary phase. ADH II mRNA began to accumulate at about 14 h, a time which was coincident with the time of increase in the rate of ADH II protein synthesis shown in Fig. 7a.

**ADH I mRNA Levels as Detected by Hybridization**—The preceding analysis only detected functional ADH I mRNA. In order to quantitate ADH I mRNA irrespective of its ability to be translated in vitro, total ADH I RNA was detected by hybridization to the cloned ADCl (ADH I) gene (20). The interpretation of these analyses is not straightforward because the ADCl and ADR2 (ADH II) genes are 90% homologous in nucleotide sequence (23, 29), and no hybridization conditions were found which allowed the ADCl gene to hybridize solely to ADH I mRNA. Nonetheless, the quantitation of ADH I mRNA hybridization was made possible, as shown below, by the fact that each respective ADH gene hybridized about 6-fold better to its homologous mRNA than to the heterologous mRNA.

In the first experiment, samples of total yeast RNA were analyzed which contained primarily only one or the other of the ADH mRNAs as detected by the in vitro translation assay. The RNAs which were chosen were extracted from strain 358-21 12 and 26 h after growth on glucose-containing medium (see Fig. 7b, Miniprint). These were the respective times when only ADH I mRNA was detected and when ADH II mRNA was in 20-fold excess over the amount of ADH I mRNA that was present. Total RNA was fractionated by electrophoresis on agarose gels after glyoxylation (24). The RNA was blotted from the gel into nitrocellulose (21) and hybridized to nick-translated fragments of either ADCl or ADR2. The resultant autoradiograms are depicted in the inset in Fig. 8 (see lanes a and b, Miniprint). Lanes a and b of the upper part of the inset show the hybridization of the ADCl gene to the yeast RNA samples which contained primarily ADH I or ADH II, respectively, as determined by in vitro translation. Although equal amounts of ADH mRNA were present in each sample, as assayed by the in vitro translation assay, the ADCl gene hybridized about 6-fold better to the sample containing the ADH I mRNA than to that containing the ADH II mRNA as ascertained after densitometric analysis of the autoradiograms. When the ADR2 gene was used as the hybridization probe, it hybridized about 6-fold better to the sample containing ADH II mRNA (lower part of inset, lane b) than to that containing primarily ADH I mRNA (lower, lane a). Furthermore, the sizes of ADH RNA species in the two samples confirm the identity of the RNAs since S1 endonuclease assays indicate that ADH I mRNA is smaller (30 base pairs) than ADH II mRNA (23, 29). As seen in lane a of the inset in Fig. 8, the RNA (identified above as ADH I) which hybridized best to the ADCl gene is slightly smaller than the RNA in lane b of the inset (identified as ADH II) which hybridized best to the ADR2 gene. These results confirm that ADH I mRNA declines when yeast are grown into stationary phase, indicating that ADH I mRNA is not present at a constant concentration irrespective of growth condition.

Total ADH RNA was also analyzed during growth on ethanol-containing medium. Total yeast RNA extracted from cells that were transferred to medium containing ethanol was separated by agarose gel electrophoresis, blotted to nitrocel-lulose, and hybridized to each of the ADH genes as described above. Fig. 8, upper inset (see Miniprint) presents an autoradiogram displaying the RNAs which hybridized to the ADCl gene as a function of time after transfer. As can be seen, the total amount of ADH mRNA that hybridized to the ADCl gene declined within the first 3 h of the experiment. After 2 h, the amount of total RNA hybridizing to the ADCl gene increased. An autoradiogram displaying the RNAs which hybridized to the ADR2 gene after the transfer to ethanol-containing medium is presented in the lower section of the inset in Fig. 8. These results are presented quantitatively in Fig. 8. The amount of each ADH RNA present is normalized to the amount of rRNA in each sample. The amount of each ADH RNA was determined after assuming that each gene hybridized 6-fold better to the homologous RNA than to the heterologous mRNA (see above) and by assuming based on S1 endonuclease assays (29) that no ADH II RNA was present at the zero time point. As shown in Fig. 8, the amount of ADH I mRNA declined 7-fold within the first 3 h after transfer to medium containing ethanol. Both the kinetics of ADH I mRNA decrease and the kinetics of ADH II mRNA increase were identical with those found for the mRNA levels as measured in the in vitro translation system (Fig. 3b, Miniprint).

**DISCUSSION**

Previous studies on the regulation of ADH I dealt with its activity as analyzed by starch gel electrophoresis (3, 5). From these studies, it was concluded that ADH I was synthesized constitutively. The results reported here have shown, however, that ADH I is not a constitutive enzyme: the amount of total and functional mRNA coding for ADH I and the rate of ADH I protein synthesis declined 6- to 10-fold after transfer to a nonfermentable carbon source or during growth into stationary phase. Yet, after this decline, ADH I protein synthesis remained at 0.5-1.0% of total yeast protein synthesis. Since ADH I was shown not to turn over rapidly, even this reduced rate of its synthesis during growth on a nonfermentable carbon source or during stationary phase would maintain a relatively high level of ADH I activity (i.e. the appearance of its being synthesized constitutively). Thus, the results reported here are not inconsistent with the previous studies. They do indicate, although, the limitation in applying the word "constitutive" to enzymes whose actual rates of protein synthesis have not been obtained.

The data presented in this paper suggest that the ADCl gene is transcriptionally regulated. Recent studies using an endonuclease S1 protection assay also show that total ADH I mRNA declines during growth on ethanol-containing medium (29). In addition, when the ADCl promoter is placed in front of the yeast cytochrome c structural gene, cytochrome c mRNA declines during growth on ethanol-containing medium, implying that the amount of transcription from the

\* A. Sledziewski, personal communication.
ADCl promoter decreases during growth on ethanol-containing medium.

The recent finding that the mRNA levels for the fermentative enolase also decrease during growth on nonfermentable carbon sources (28) is suggestive that all the glycolytic enzymes may be regulated. Attempts by us to isolate regulatory mutants which control ADH I synthesis have proven unsuccessful. One mutant, gerl, which affects several glycolytic enzymes has been described, but its effect on ADH I has not been investigated (11). However, it has been shown to depress the mRNA levels of several other glycolytic enzymes.5

The results in this paper also indicate that the ADH II mRNA is not present during growth on glucose-containing medium, a result which has been confirmed by an S1 endonuclease assay (29). It should be noticed that the amount of ADH II mRNA and its rate of protein synthesis is much greater during natural depletion of glucose from the medium (diauxic growth) than during growth on ethanol-containing medium. Whether ADH I and ADH II are coordinately regulated in an inverse fashion cannot be ascertained, although regulatory genes affecting ADH II mRNA expression appear to have no effect on ADH I expression (32).

The size of the ADH I and ADH II mRNAs as measured after agarose gel electrophoresis (1.2 kilobases) conforms to the size as determined by S1-mapping (29). A larger, less abundant RNA also hybridized to the ADCl gene. This RNA species is found during ethanol growth conditions and has been identified as an ADClI transcript commencing 1.2 kilobases in front of the ADCl gene (31). This larger transcript presumably is capable of being translated into an ADH I gene product, for an RNA twice the size of the normal ADH I mRNA has been found to make an ADH I protein in the wheat germ cell-free system.6

While the gluconeogenic and fermentative reversible reactions in yeast are considered to be catalyzed by the same enzyme, the case of the ADH isozymes indicates that different enzymes for the same reaction may be expressed depending on the needs of the cell. This would occur in much the same way and for a similar purpose as for the classical lactate dehydrogenase isozymes in mammals. Kinetic characteristics of one enzyme would make it more effective for the gluconeogenic pathway whereas a different enzyme would be used for the reverse fermentative pathway. The kinetic parameters of the ADH isozymes have been found to be consistent with their expression (4). The existence in yeast of multiple genes and/or multiple proteins of glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase, aldolase, phosphoglucone isomerase, enolase, and hexokinase suggests that this multiplicity serves this function (12-17). Recent evidence that the enolase genes are differentially expressed depending on the available carbon source lends additional credence to this view (28). Similar findings have been presented for the two hexokinase isozymes (13, 14). This type of general enzyme usage for reversible reactions would add another level of control over the gluconeogenic and fermentative pathways in yeast other than through the regulation of the irreversible reactions and the flux of substrates through the pathways. Determination of the kinetic parameters of these other isozyme pairs for both the forward and backward reactions which they catalyze would be instrumental in substantiating this pattern.

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5 M. J. Holland, personal communication.
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Regulation of Yeast Alcohol Dehydrogenase I

Experimental Procedures

Methods

Stereisolation of yeast alcohol dehydrogenase was conducted as described previously (9). The purified enzyme was used in all experiments. The enzyme was stored at -20°C in a solution containing 50% glycerol and 50 mM potassium phosphate buffer, pH 7.4. The enzyme was dialyzed against the same buffer before use.

Pulse-labeling of yeast alcohol dehydrogenase

The enzyme was isolated from a strain of yeast that had been grown for 24 hours in a medium containing 2% glucose and 1% galactose. The enzyme was then labeled with [3H]methionine and [3H]cysteine as described previously (9). The enzyme was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography.

Preparation of yeast alcohol dehydrogenase for immunoprecipitation

The enzyme was isolated from a strain of yeast that had been grown for 24 hours in a medium containing 2% glucose and 1% galactose. The enzyme was then labeled with [3H]methionine and [3H]cysteine as described previously (9). The enzyme was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography.

Figure 1. Total protein synthesis and ADH protein synthesis after pulse-labeling with radioactive methionine. The results are expressed as the percentage of total protein synthesis and ADH protein synthesis in the yeast cell-free system. RNA was extracted from strain 25-125 at time zero and at various times after transfer to medium containing ethanol. The results are expressed as the percentage of total protein synthesis and ADH protein synthesis in the yeast cell-free system. RNA was extracted from strain 25-125 at time zero and at various times after transfer to medium containing ethanol.

Figure 2. Total protein synthesis and ADH protein synthesis after pulse-labeling with radioactive methionine. The results are expressed as the percentage of total protein synthesis and ADH protein synthesis in the yeast cell-free system. RNA was extracted from strain 25-125 at time zero and at various times after transfer to medium containing ethanol. The results are expressed as the percentage of total protein synthesis and ADH protein synthesis in the yeast cell-free system. RNA was extracted from strain 25-125 at time zero and at various times after transfer to medium containing ethanol.
Figure 7. ADH protein synthesis and mRNA activity in strain 350-21 during diauxic growth.

The results in a are expressed as the percentage of total protein synthesis in cells which lack ADH protein synthesis and in b are expressed as the percentage of total protein synthesis in the absence of the ADH gene in the cell-free system. Pulse-labelings were for 15 min at the times indicated.

Glucose became depleted between 12 and 14 hr. --- , ADH; - - - - , ADH-lac.

Figure 8. Quantitation of ADH mRNAs by hybridization.

Total yeast RNA was denatured and fractionated in agarose gels (20) as described (21). Hybridization of cloned DNA to the nitrocellulose blots was conducted as described in Experimental Procedures. All hybridizations were performed at 55°C in a solution containing 5 x SSPE, 5 x Denhardt's solution, 0.1% SDS, and 0.1% BSA. After hybridization, the blot was washed three times in 2 x SSPE, 0.1% SDS, 0.1% BSA at 55°C for 15 min each wash. The blots were then autoradiographed and exposed for 2-3 days.

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It was also assumed that if 0 hr no ADH mRNA existed, which was confirmed by 32P-end-labeling of RNA in each sample. The amount of ADH and ADH-lac mRNA were calculated by assuming that the respective ADH DNA hybridized six times as well as its homologous RNA as to the heterologous RNA (see text).