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Translational control of catalase synthesis by hemin in the yeast *Saccharomyces cerevisiae*

(catalase T/catalase A/homologous mRNA-dependent translation system/heme-deficient *ole3* mutant)

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ABSTRACT **mRNA-dependent cell-free protein synthesis systems were prepared from a heme-deficient *ole3* mutant of the yeast *Saccharomyces cerevisiae* grown either in the absence or in the presence of the heme precursor δ-aminolevulinate. When supplemented with total yeast mRNA, the two systems—from heme-deficient and from heme-containing cells—translate most mRNAs with comparable efficiencies. mRNAs coding for the hemoproteins catalase T and catalase A, however, are translated at a low rate by the system from heme-deficient cells whereas their translation in the system from heme-containing cells is comparable to that observed in a heterologous *in vitro* system from wheat germ. Addition of 10 μM hemin to the system from heme-deficient cells stimulates translation of catalase mRNAs significantly. Control experiments showed that the results obtained cannot be explained by specific proteinase or nuclease action. Together with previous findings indicating lack of translation of catalase T mRNA in heme-deficient cells *in vivo*, the results demonstrate that specific control of yeast catalase formation occurs at the level of translation.**

Although the regulation of gene expression at the level of transcription appears to be of primary importance in eukaryotic as well as prokaryotic organisms, there is growing evidence that translational control has great significance as an additional mechanism affecting the level of final gene products. General control of mRNA translation by hemin‡ and by double-stranded RNA has been described; in particular, hemin control of protein synthesis in reticulocyte lysates has been studied extensively (1–3). In a number of cases, it has been possible to demonstrate specific control of translation of individual mRNAs or certain groups of mRNAs. Competition between cellular and viral mRNAs has been studied (4–6). mRNA selection has also been observed during differentiation of *Dictyostelium* (7) and in the course of development of the surf clam *Spisula solidissima* (8). More recently, mRNA discrimination induced by heat shock in *Drosophila* (9, 10) and specific control of translation of procollagen mRNA (11) have been studied in cell-free translation systems.

Little is known about mechanisms involved in specific translational control. The isolation of mRNA-dependent cell-free translation systems from spheroplasts of the yeast *Saccharomyces cerevisiae* (12) and from whole yeast cells (13) has been reported recently. Especially the latter system can be isolated from various yeast strains grown under different physiological conditions and is therefore well suited for translational control studies. Because both *in vivo* and *in vitro* methods as well as genetic techniques are available for studies with yeast, this lower eukaryotic organism appears to be particularly attractive for investigating the mechanism and importance of translational control in the regulation of specific genes.

Heme has been shown to act as a regulator of gene expression in a number of organisms. Besides its role in translational control in reticulocytes (1–3) it also participates in the induction of globin mRNA and in erythroid differentiation (14–18). Recently it was reported that hemin enhances the differentiation of mouse 3T3 cells to adipocytes (19). Control of δ-aminolevulinate synthase formation by heme has been observed and has been postulated to occur at the transcriptional level (20). In *S. cerevisiae*, heme regulates the levels of mRNAs for at least two hemoproteins, catalase T (21) and iso-1-cytochrome *c* (unpublished data). Furthermore, post-transcriptional control of catalase T formation by heme has recently been demonstrated (22).

This paper describes the analysis of this post-transcriptional control with the help of homologous cell-free translation systems. These were isolated from a heme-deficient *ole3* mutant (23) grown either in the presence or in the absence of the heme precursor δ-aminolevulinate. The results show that the discrimination against catalase mRNA previously observed *in vivo* (ref. 22; unpublished data) also occurs in the cell-free system isolated from the *ole3* mutant grown in the absence of heme and that this discrimination can be relieved, at least partly, by supplementation of the translation system with hemin.

MATERIALS AND METHODS

Growth of Yeast Strains. *S. cerevisiae* strain DczH1-1B (α *ole3 leu1*) (22, 23) was used for the isolation of cell-free translation systems. Cells were grown at 28°C in batches of 3–4 liters in a New Brunswick fermenter (Microferm) in a medium containing 2% glucose, 1% yeast extract, 1% bactopeptone, Tween 80 (2.6 g/liter), and ergosterol (12 mg/liter), in the presence or absence of the heme precursor δ-aminolevulinic acid (50 mg/liter). Cultures were harvested when the culture had reached an optical density (660 nm) of 1.0. Strain D273-10B (α) (ATCC 24657) was used as source of total yeast mRNA. To obtain a mRNA fraction rich in catalase mRNAs (24), cells were grown anaerobically and then adapted to oxygen for 45 min as described (25).

Preparation of mRNA-Dependent Translation Systems. The translation systems used were prepared, as described (13), by mechanical breakage of yeast cells, isolation of a 30,000 × *g* supernatant fraction, fractionation with Sephadex G-25, and treatment with micrococcal nuclease.

Isolation and Translation of Yeast mRNA. Total yeast mRNA was isolated as described (21). It was translated in yeast *in vitro*

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‡ The term "hemin" (Fe^{3+}-protoporphyrin IX) is used where the effects of this compound were studied *in vitro*. In cases in which the species playing a role cannot be specified, the term "heme" is used.

protein synthesis systems in 100-μl incubation mixtures containing 19 protein amino acids (50 μM; methionine excluded), 25 μCi (1 Ci = 3.7×10^{10} becquerels) of L-[^{35}S]methionine (600 Ci/mmol; Amersham), 2.5 mM magnesium acetate, 220 mM potassium acetate, 38 mM Hepes/KOH at pH 7.4, 2.7 mM dithiothreitol, 0.5 mM ATP, 0.1 mM GTP, 20 mM creatine phosphate, 2 μg of creatine kinase (Boehringer Mannheim), 2 mM glucose 6-phosphate, 50 μg of total yeast RNA, and 60 μl of nuclease-treated 30,000 $\times$ *g* supernatant fraction (13). Incubations were carried out for 90 min at 20°C. Incorporation of label into proteins was assayed by trichloroacetic acid precipitation (26). Incorporation into catalase T and catalase A was monitored by immunoadsorption (27) of the proteins with the help of specific antisera (25), separation of products immunoadsorbed by dodecyl sulfate/polyacrylamide gel electrophoresis (28), visualization of labeled proteins by fluorography (29), and by liquid scintillation counting of catalase bands cut from the gels. Total products of translation were precipitated with cold 25% (wt/vol) trichloroacetic acid in the presence of 0.01% deoxycholate and were then characterized by two-dimensional separation using a modification of the procedure of O'Farrell (30) according to Garrels (31). Molecular weight standards used were phosphorylase *b* (92,000), bovine serum albumin (69,000), ovalbumin (46,000), and carbonic anhydrase (30,000).

RESULTS

In an attempt to set up an *in vitro* assay for heme control of catalase mRNA translation, mRNA-dependent cell-free translation systems were isolated from the *S. cerevisiae* mutant DczH1-1B (*ole3*). This mutant is heme-deficient because of a specific block in the synthesis of the heme precursor δ-aminolevulinate. When grown in the presence of δ-aminolevulinate, its respiration is close to normal (32). Cell-free translation systems were isolated from the mutant after growth either in the presence or in the absence of δ-aminolevulinate. Systems from both cultures efficiently translate total yeast mRNA (13). In the experiments described in this paper, a 100-μl incubation mixture containing the system from cells supplemented with δ-aminolevulinate incorporated approximately 1.5×10^7 cpm of L-[^{35}S]methionine (600 Ci/mmol) into trichloroacetic acid-precipitable material. Systems from heme-deficient cells had 60–80% of the protein-synthesizing activity of the system from supplemented cells.

Fig. 1 illustrates the capacity of the two systems isolated from heme-containing and heme-deficient cells to translate catalase mRNAs contained in total yeast mRNA fractions. Quantitative evaluation of these results is summarized in Table 1. The capacity of the system from δ-aminolevulinate-supplemented cells (S1) to translate catalase mRNAs was similar to that observed previously with a translation system from wild-type yeast cells (13) or with a protein synthesis system from wheat germ (24). However, when the same total mRNA fraction from wild-type yeast cells was translated in the system from heme-deficient cells (S2), synthesis of catalase T and catalase A was much lower compared to total protein synthesized. The capacity of the system from heme-deficient cells to synthesize catalase T and catalase A was increased significantly by the addition of hemin to the incubation mixture, whereas this compound had little effect on the general rate of protein synthesis. No further stimulation of catalase synthesis was observed when hemin was added to the system from δ-aminolevulinate-supplemented cells (not shown).

In some experiments, a second band was observed slightly below the band migrating like authentic catalase T (see especially lanes 2 and 3 in Fig. 1). The intensity of this band showed little if any dependence on hemin. In quantitative evaluation, only the radioactivity present in the catalase T (upper) band was counted. Additional unidentified bands in lane 3 appear to have been stimulated by hemin. However, this effect, which never occurred in the case of catalase A, was only observed occasionally whereas a 2- to 4-fold stimulation of catalase T formation by addition of hemin (see also Fig. 2) was consistently found in many experiments. Maximal stimulation of catalase T formation was obtained by addition of 5–10 μM hemin (Fig. 2). No stimulation of catalase synthesis was detected when the buffer used to dissolve hemin was added to the incubation mixture or when hemin was added at the end of the incubation period.

Our results strongly indicate that the discrimination against catalase mRNA observed in heme-deficient cells can be repro-

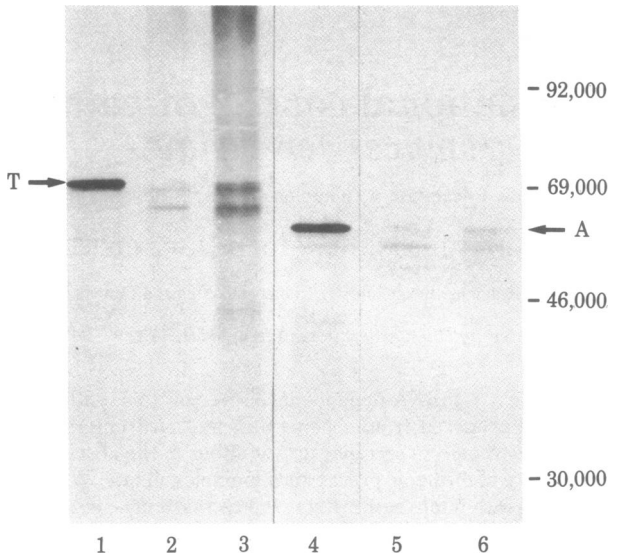

FIG. 1. Catalase formation by yeast translation systems. Total yeast mRNA was translated in the presence of L-[^{35}S]methionine in translation systems from *ole3* mutant cells grown in the presence of δ-aminolevulinate (lanes 1 and 4) or in the absence of this heme precursor (lanes 2, 3, 5, and 6). Hemin (10 μM) was added to some incubation mixtures from a stock solution (1 mM) in 90% (vol/vol) ethylene glycol/20 mM Hepes/KOH, pH 8.2 (lanes 3 and 6). Catalase T (lanes 1, 2, and 3) and catalase A (lanes 4, 5, and 6) were isolated from incubation mixtures by immunoadsorption, separated by polyacrylamide gel electrophoresis (10% gels), and visualized by fluorography. T, position of catalase T; A, position of catalase A (labeled *in vivo*).

Table 1. Capacity of yeast translation systems to synthesize catalase T and catalase A

System	Trichloroacetic acid precipitate immunoadsorbed, % $\times 10^3$	
	As catalase T	As catalase A
S1	48.0	11.5
S2	3.8	2.2
S2/10 μM hemin	13.0	3.8
S1/S2 (75:25)	25.7	8.2
S1/S2 (50:50)	15.9	6.2
S1/S2 (25:75)	9.0	3.7

Total yeast mRNA was translated in systems from cells grown in the presence (S1) or absence (S2) of δ-aminolevulinate. L-[^{35}S]Methionine-labeled catalase proteins synthesized were isolated by immunoadsorption followed by dodecyl sulfate/polyacrylamide gel electrophoresis. Catalase bands detected by fluorography were cut from gels and assayed in a liquid scintillation counter. Incorporation of L-[^{35}S]methionine into total protein was assayed by trichloroacetic acid precipitation.

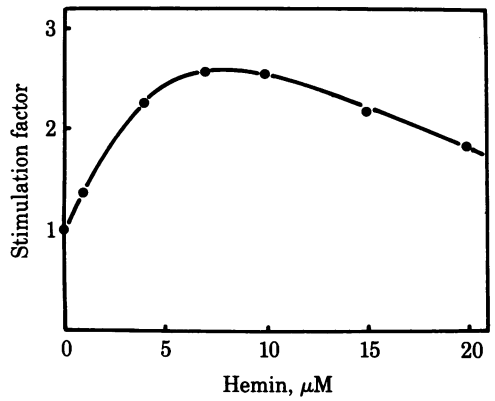

FIG. 2. Hemin stimulation of catalase T synthesis. Total yeast mRNA was translated in the presence of L-[^{35}S]methionine in a translation system from heme-deficient *ole3* mutant cells. Various concentrations of hemin were added to incubation mixtures. Catalase T synthesis was assayed by immunoadsorption, dodecyl sulfate/polyacrylamide gel electrophoresis, fluorography, and counting of radioactivity in catalase T bands.

duced in a cell-free translation system. Further experiments were carried out to characterize the specificity of the effect observed and to exclude possible artifacts.

Two-dimensional separation of *in vitro* translation products of total yeast mRNA showed that the heme effect observed in the case of catalases was remarkably specific (Fig. 3). Although some differences could be detected by comparison of the patterns of main products synthesized by the systems from heme-deficient and heme-containing cells, synthesis of the majority of translation products of total yeast mRNA by the two systems seemed to occur with comparable efficiencies. In those cases in which quantitative differences were observed it could not be clarified unambiguously whether these were genuine or were experimental artifacts. It seems possible that the effect of heme on translation is not restricted to catalase mRNAs. In addition, like catalases, which could not be detected on the two-dimensional gels shown in Fig. 3 because of the low levels of their mRNAs in yeast cells, other minor proteins also may be affected.

Although this seemed unlikely because of the stimulation of catalase synthesis by addition of hemin, two further experiments were carried out to investigate whether the low amount of catalase obtained with the system from heme-deficient cells might possibly have resulted from such systems containing either a nuclease, which degraded catalase mRNAs *in vitro* with fairly high specificity, or a proteinase, which specifically hydrolyzed newly synthesized catalase proteins. When extracts of heme-deficient cells were mixed in various proportions with those from cells supplemented with δ-aminolevulinate the synthetic capacity for catalases was not inordinately affected (Table 1).

To obtain a more reliable estimate of the possible interference of proteinases or nucleases with catalase accumulation during translation *in vitro*, we attempted to block specifically the translational activity of the system from heme-deficient cells while leaving any proteinases or nucleases unaffected. When mixed with an active system, such an extract should still retain its hypothetical degradative activity without contributing to the protein synthesis activity of the mixture. A system thus treated therefore should be more suitable for detecting any degradative activity than one hypothetically exhibiting both catalase synthesis and degradative activities.

To inhibit translational activity, the ribosomes present in system S2 from heme-deficient cells were inactivated by incubation with EDTA. When an equimolar amount of Mg^{2+} was added to such a system before it was tested for translational activity in the usual way, most of the ribosomes were not reactivated and its protein synthesis activity was <5% of the original value. When inactivated system S2 was mixed with active system S1, catalase T synthesis was not significantly inhibited beyond a general reduction of protein synthesis caused by the

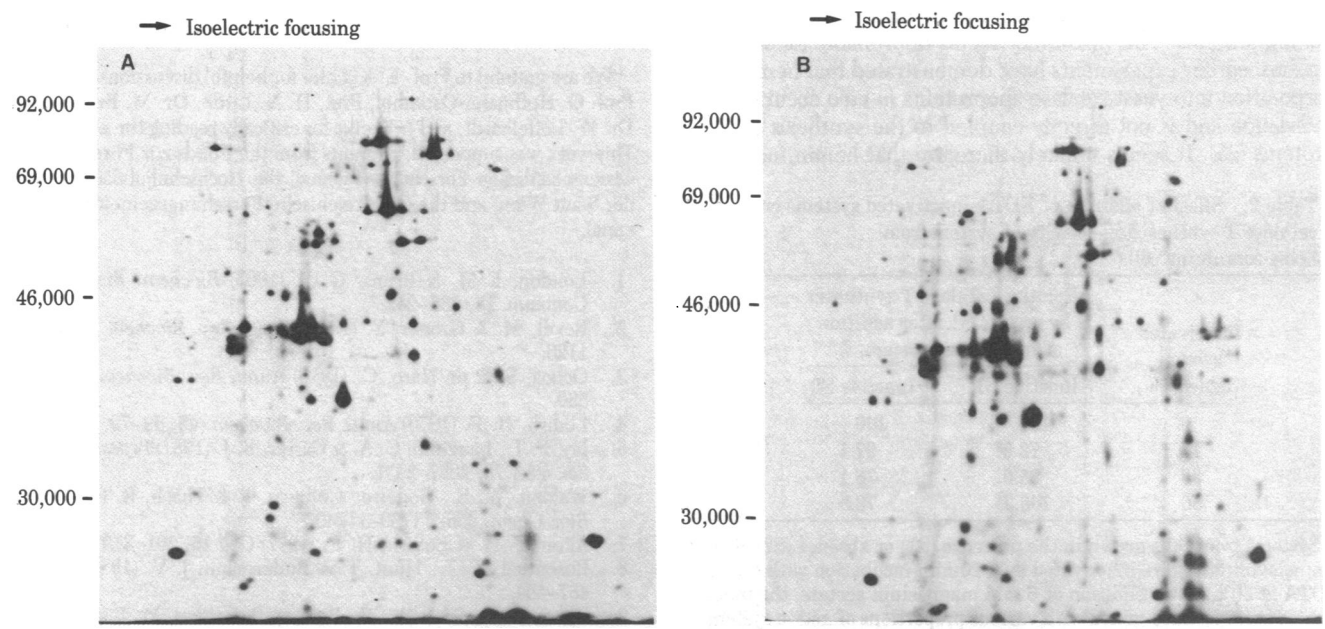

FIG. 3. Two-dimensional separation of proteins synthesized by system from heme-deficient cells (*A*) and from cells supplemented with δ-aminolevulinate (*B*). Incubation mixtures (100 μl) were treated with 25% trichloroacetic acid/0.01% deoxycholate; the precipitates were washed twice with 2% trichloroacetic acid and twice with acetone. They were then solubilized by boiling for 3 min in 2% (wt/vol) sodium dodecyl sulfate/100 mM Tris·HCl, pH 6.8/20% (vol/vol) glycerol/1 mM EDTA/10 mM dithiothreitol. After boiling, urea was added to the samples (final concentration, 10 M) and proteins were separated in the first dimension by isoelectric focusing (pH 4.0–8.5) followed by dodecyl sulfate/polyacrylamide gel electrophoresis (10% acrylamide) in the second dimension. ^{35}S-Labeled proteins were visualized by fluorography (exposure time, 2 days).

addition of the EDTA-treated extract (Table 2). In addition, the results of such experiments did not differ significantly from those obtained in a control experiment in which active system S1 was mixed with EDTA-inactivated S1.

Furthermore, it has been calculated that even if the slight decrease in catalase synthesis observed in some mixtures were due to the presence of a specific proteinase or nuclease, this effect would be much too small to explain the low capacity of the active heme-deficient system S2 to synthesize catalase. We consider it fairly unlikely that a proteinase or a nuclease possibly present in the system from heme-deficient cells is inactivated irreversibly by the EDTA treatment. Together with the data showing hemin stimulation of catalase mRNA translation, the results obtained in the experiment strongly indicate that the low capacity of the translation system from heme-deficient cells to synthesize catalases is not due to degradation of catalase mRNAs by a nuclease or degradation of catalase proteins or nascent catalase polypeptide chains by a proteinase.

DISCUSSION

In agreement with earlier results obtained in studies *in vivo*, the *in vitro* experiments described in this paper provide more direct evidence for the occurrence of translational control by discrimination against catalase mRNAs in the absence of heme. (*i*) Strong discrimination was observed in a system isolated from heme-deficient cells, and this discrimination was partly compensated for by addition of hemin to translation mixtures. (*ii*) The effect observed was remarkably specific. (*iii*) Mixing experiments have shown that the apparent low rate of catalase formation by the system from heme-deficient cells cannot be explained by specific degradation of catalase protein or mRNA.

It seems necessary to consider, however, whether the effects observed could be explained by formation of different products by the two systems—namely, catalase apoproteins by the hemin-deficient system and catalase proteins containing hemin by the system from heme-containing cells. If this were true, proteolytic enzymes present in both systems might degrade catalase apoproteins to a greater extent than the hemin-containing proteins. This possibility seems improbable for several reasons: earlier experiments have demonstrated that hemin incorporation into yeast catalase apoproteins *in vivo* occurs after translation and is not directly coupled to the synthesis of the proteins (25). It seems unlikely therefore that hemin incorporation into catalase proteins newly synthesized by an homologous *in vitro* protein synthesis system will occur with sufficient efficiency to provide any significant protection against proteolytic attack. However, if a major part of the catalase proteins synthesized by the system from heme-containing cells did incorporate hemin, such an effect would have to be dominant in mixing experiments if hemin is present in excess in the extract. Alternatively, if hemin is limiting in the system from heme-containing cells, catalase accumulation should be stimulated by addition of this compound. The results of our mixing experiments show no dominance of the system from heme-containing cells. Instead, a weak dominance of the heme-deficient system could be deduced from the data. Furthermore, catalase formation by the system from heme-containing cells is not stimulated by addition of hemin.

Table 2. Effect of addition of EDTA-inactivated systems on catalase T-synthesizing activity of system from heme-containing cells

Inactivated system added, %	Relative catalase T synthesis by system S1 after addition of inactivated system, %*	
	Inactive S1	Inactive S2
0	100	100
17	72.5	97.1
33	85.0	78.1
50	106.2	73.5

Systems from cells grown in the presence (S1) or absence (S2) of δ-aminolevulinate were inactivated by a 20-min incubation with 6 mM EDTA at 20°C. After addition of 6 mM magnesium acetate, the inactivated systems were mixed with various proportions of active system S1. Translations were carried out in a total volume of 100 μl in the presence of L-[^{35}S]methionine (25 μCi), total yeast mRNA, and a constant volume (60 μl) of the mixtures of active and inactivated systems. Catalase T synthesized was assayed by immunoadsorption.

* Catalase T-synthesizing activity was calculated as fraction of trichloroacetic acid-precipitable radioactivity and is presented as percentage of the activity of the undiluted system S1.

Further studies are necessary to clarify the mechanism of translational control of catalase formation. The results obtained are consistent with the hypothesis that a rate-limiting factor stimulating translation of catalase mRNAs is inactive in a translation system isolated from heme-deficient cells but is activated by hemin supplementation of the cell-free system. Other explanations are possible, however, and further experiments will also have to clarify whether initiation or a later step of catalase mRNA translation is controlled. It also remains to be investigated whether more than a formal analogy exists between the specific hemin control of catalase mRNA translation in *S. cerevisiae* and the unspecific hemin control of translation observed in reticulocytes (1–3).

As in the case of *Drosophila* heat shock proteins (9), gene expression has now been shown in the case of yeast catalase to be regulated by control not only of concentrations of mRNAs but also of their translation. It can be speculated that simultaneous control of gene expression at two levels may be a fairly common phenomenon providing additional regulatory flexibility to the cell. The experimental system developed to study catalase regulation can be used to test a more general occurrence of simultaneous transcriptional and translational control in yeast and will allow a more detailed study of molecular mechanisms involved in specific regulation on the level of translation.

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