CELL-FREE SYNTHESIS OF *SACCHAROMYCES CEREVISIAE* CATALASE T

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1. Introduction

Little is known about the nature of primary translation products of cell organelle proteins synthesized in the cytoplasm or about the mechanisms involved in specific transport of such proteins to their sites of function, although some important progress has recently been made in this direction [1–4]. There is also a scarcity of information on the control of intracellular levels of such proteins. In most cases it is unknown whether levels of eukaryotic proteins are regulated by control of messenger RNA synthesis, translation or protein degradation.

Studies on the biosynthesis of yeast catalases were designed to contribute to our understanding of these problems. The yeast *Saccharomyces cerevisiae* contains catalase T and A, both tetrameric hemoproteins [5,6]. Their biosynthesis in vivo and its regulation by oxygen [7–9] and glucose [10] have been investigated. In connection with these studies, in vitro translation of catalase mRNAs should yield information about the nature of primary translation products and about the presence of catalase mRNAs in cells containing no catalase proteins, e.g., in anaerobic yeast [7,8] or in heme-deficient mutants (W. Woloszczuk, D. B. Sprinson, H.R., in preparation).

This paper contains the first results of the investigation and reports on the translation of catalase T mRNA in cell-free systems from wheat germ and from rabbit reticulocytes. While this work was in progress, results of similar studies on rat liver catalase have been published [11,12].

Dedicated to Professor O. E. Polansky on the occasion of his 60th birthday

2. Materials and methods

2.1. *Growth of cells*

The haploid *Saccharomyces cerevisiae* strain D 273-10B (ATCC 24657) was used in all experiments. Cells were grown on a semisynthetic medium [13] containing 0.3% glucose. Cells harvested between early logarithmic and early stationary phase were successfully used for isolation of mRNA. For in vivo labelling of proteins, cells were grown as above, but [^{35}S]methionine (10 μCi/ml, 800 Ci/mmol) was added to the culture 3 h before harvesting the cells.

2.2. *Isolation of RNA*

Cycloheximide (100 μg/ml) was added to the cultures 5 min before cells were harvested. Then the culture was cooled rapidly on ice, the cells were centrifuged and washed twice with a medium containing 10 mM Tris–HCl, 10 mM Mg acetate, 10 mM KCl and 50 μg cycloheximide/ml (pH 7.5). Then the cells were disrupted with glass beads [14] in a 1:1 mixture of 0.1 M Tris–HCl, 0.2 M LiCl, 0.2% sodium dodecylsulfate, 1 mM EDTA (pH 7.5) and phenol/chloroform/isoamylalcohol (50:50:1). The glass beads were rinsed with the same mixture, and the combined extracts were centrifuged for 10 min at 12 000 rev./min in a Sorvall SS 34 rotor. The aqueous phase obtained was extracted twice with phenol/chloroform/isoamylalcohol and once with chloroform.

Sodium acetate (final conc. 0.2 M, pH 5.0) was added to the aqueous solution and the RNA was precipitated overnight with 2 vol. of ethanol at −20°C. The precipitate was washed with 70% ethanol, dried in a desiccator and dissolved in sterile H_2O. The RNA was then reprecipitated for 60 min at −20°C with an equal volume 6 M sodium acetate (pH 5.0), washed with 70% ethanol and dried as above.

Poly(A)-containing RNA was isolated from total RNA by poly(U)–Sepharose chromatography [15].

2.3. *In vitro translation of RNA*

Wheat germ S30 fractions [16] were prepared without the pre-incubation step. Translation was carried out at 20°C for 150 min. Incubation mixtures (50 μl) contained 20 μl S30, 20 mM Hepes (pH 7.6, adjusted with KOH), 2 mM dithiothreitol, 1 mM ATP, 0.2 mM GTP, 8 mM creatine phosphate, 50 μg/ml creatine kinase, 108 mM KCl, 2 mM Mg acetate, 50 μM spermine, 50 μM of each of the unlabelled protein amino acids and 10 μCi [^{35}S]methionine (800–1100 Ci/mmol). Total RNA (20 μg) or poly(A)-RNA (3 μg) were added.

A rabbit reticulocyte lysate treated with micrococcal nuclease was prepared and translation in this system were carried out as in [17].

2.4. *Immunoprecipitations*

Rabbit antiserum (gammaglobulin fractions) specific for yeast catalase T was kindly supplied by Mag. E. Hartter from our laboratory. Antiserum directed against yeast triosephosphate dehydrogenase was donated to us by Dr G. Schatz (Biozentrum der Universität Basel). Immunoprecipitation of catalase T labelled in vivo was carried out as in [7].

Immediately after in vitro translation, sodium dodecylsulfate (final conc. 2–4%) was added to incubation mixtures, which were then kept in a boiling water bath for 2 min. They were then diluted to 0.2–0.4% dodecylsulfate with 25 mM veronal, 0.15 M NaCl, 1% Triton X-100 (pH 8.2), antiserum (1–5 μl) was added and the mixture kept at room temperature overnight [4]. A 10% suspension (20 μl) of *Staphylococcus aureus* cells, treated as in [18], in veronal/NaCl/Triton X-100/0.1% dodecylsulfate, were added for 1 h at room temperature. The suspension of bacteria was then centrifuged and the pellet was washed 3 times with veronal/NaCl/Triton X-100/0.1% dodecylsulfate. Finally, the adsorbed material was eluted by boiling for 4 min with 2.5% dodecylsulfate, 10 mM dithiothreitol.

2.5. *Polyacrylamide gel electrophoresis*

Samples were analyzed on 7.5% polyacrylamide gels as in [19]. Fluorography was carried out as in [20], but dimethylsulfoxide was replaced by acetic acid.

2.6. *Materials*

[^{35}S]Methionine was purchased from the Radiochemical Center, Amersham. Creatine kinase, ATP, GTP and creatine phosphate were from Boehringer, Mannheim, poly(U)–Sepharose 4B was from Pharmacia, Uppsala. Wheat germ (Niblack N. Foods, Rochester, NY) was a generous gift by Dr G. Kreil. The Cowan I strain of *Staphylococcus aureus* was kindly given to us by Dr G. Fink.

3. Results

Total RNA and poly(A)-RNA obtained from whole yeast cells grown to logarithmic or early stationary growth phase was tested in the wheat germ system for the presence of active mRNA. Similar results were obtained with preparations from cells harvested at different growth stages. As shown in table 1, incorporation of [^{35}S]-methionine into trichloroacetic acid-precipitable material was stimulated 50–60-fold by addition of yeast RNA to the incubation system. For successful translation of proteins of higher molecular weight, incubation conditions had to be optimized in a similar way as

Table 1
Translation of yeast RNA in the wheat germ system

RNA added	cpm precipitable by trichloroacetic acid
–	3600
Total RNA (20 μg)	185 000
Poly(A)-RNA (3 μg)	230 000

Aliquots (2 μl) of 50 μl incubation mixtures were used for trichloroacetic acid precipitation [17]

for albumin [21]. Incubation temperature and concentrations of Mg^{2+} and K^+ and spermine were varied and the influence of these parameters on the efficiency of translation of proteins of different molecular weights was tested. As can be seen in fig.1, translation products up to mol. wt $>10^5$ were obtained under the conditions used.

Many attempts to obtain a specific product by immunoprecipitation with anticatalase T failed. Direct immunoprecipitation after addition of carrier, indirect immunoprecipitation with anti-gammaglobulin and immunoadsorption with *Staphylococcus aureus* cells were tried, but no specific product could be detected. Addition of various protease inhibitors during or after translation did not yield positive results either. In all these experiments, a number of unspecific bands were observed on dodecylsulfate gels, but no bands were detected in the molecular weight range of catalase monomers or of a putative precursor of the protein. Up to now, only the procedure described in section 2.4. [4] gave reproducibly positive results. In

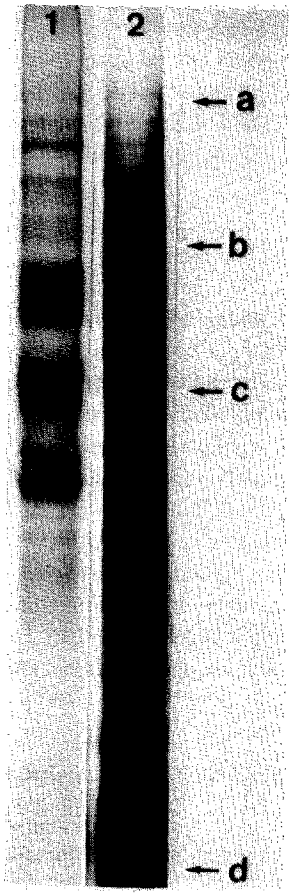

Fig.1. Dodecylsulfate gel electrophoresis of trichloroacetic acid-precipitated protein labelled in vivo (lane 1) and in vitro (lane 2). Molecular weight standards: (a) phosphorylase *a* (100 000); (b) bovine serum albumin (68 000); (c) ovalbumin (43 000); (d) egg white lysozyme (14 300).

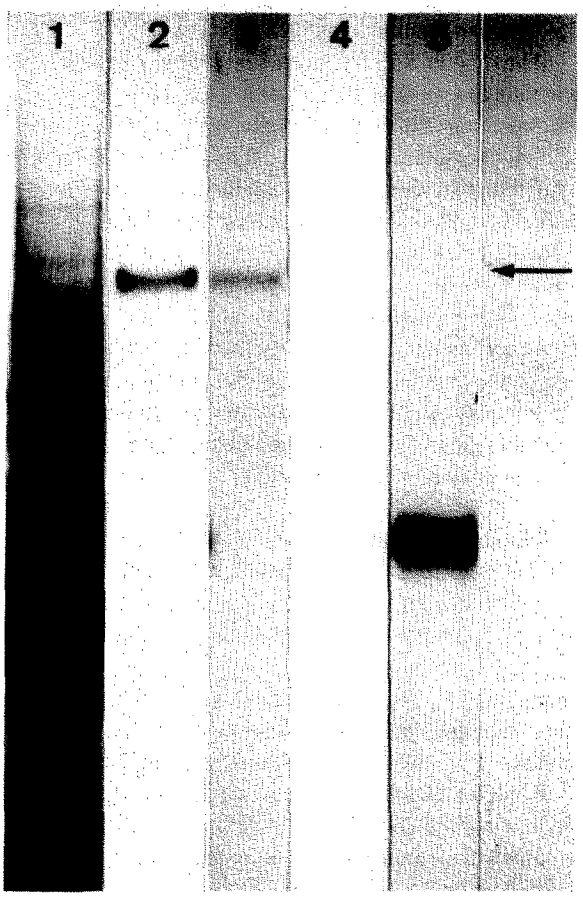

Fig.2. Dodecylsulfate gels of proteins precipitated with trichloroacetic acid or with antisera: (1) trichloroacetic acid-precipitate from wheat germ incubation with yeast poly A-RNA; (2) catalase T, labelled in vivo; (3) catalase T, immunoprecipitated from wheat germ incubation; (4) precipitation with pre-immune serum from wheat germ incubation; (5) triosephosphate dehydrogenase, immunoprecipitated from wheat germ incubation. Arrow: bovine serum albumin marker. Lanes 1, 3 and 5 were exposed for 7 days, lanes 2 and 4 for 10 days.

contrast to that, triosephosphate dehydrogenase, which was immunoprecipitated to test the reliability of our methods (fig.2, lane 5), could be precipitated specifically by a variety of procedures.

Figure 2 shows that a specific product (lane 3) with the same electrophoretic mobility as catalase T labelled in vivo (lane 2) can be obtained by immunoprecipitation from wheat germ incubation mixtures. No product was obtained by precipitation with a pre-immune serum taken from the same rabbit (lane 4) or by immunoprecipitation from incubations where yeast RNA was omitted (not shown). Only 0.005% of the total trichloroacetic acid-precipitable products was detected in the catalase T band. It is not surprising therefore that no discrete band corresponding to catalase T can be seen after electrophoretic separation of total translation products (lane 1). The same catalase T-related product was also obtained by translation of poly(A)-RNA isolated from yeast polysomes and by translation in the reticulocyte system (not shown).

5. Discussion

The results of this investigation demonstrate that catalase T mRNA from *Saccharomyces cerevisiae* can be translated in protein synthesis systems derived from wheat germ or rabbit reticulocytes. No product with similar electrophoretic properties was found in control incubations, and the product is not observed among the in vitro translation products of a yeast mutant containing no catalase T protein (in preparation). It appears at least very likely therefore that the product isolated is actually catalase T protein.

Difficulties in demonstrating formation of the specific product and the low yield of catalase T protein synthesized in vitro (0.005% of total protein compared to ~0.2% in labelling experiments in vivo) may at least partly be explained by an instability of the mRNA for catalase T against ribonucleases present in the cell-free systems or by a sensitivity of the catalase product against proteases of these systems. Remarkably, similar difficulties have been encountered [11] during translation in vitro of catalase mRNA from rat liver. Such problems may also explain why we have not yet been able to demonstrate the formation of the second catalase of *Saccharomyces cerevisiae*, catalase A, in a cell-free system.

The in vitro product detected in our experiments shows the same electrophoretic mobility as catalase T protein labelled in vivo. Analogous results have been obtained [11,12] for the peroxisomal catalase from rat liver. These authors have suggested two different interpretations for their result. It could mean that catalases are actually not synthesized in the form of precursors of higher molecular weight. Alternatively, a signal peptide normally cleaved off during intracellular transport may also be hydrolyzed by a peptidase present in the wheat germ and reticulocyte systems. Whereas these explanations may also apply to catalase T there is at least one further possibility in this case. Its subcellular localization has not yet been unequivocally clarified and it is possible that it is a cytoplasmic enzyme. Therefore, its synthesis might occur without formation of a larger precursor even if such a product of higher molecular weight exists in the case of peroxisomal catalases. Since catalase T apparently is not a glycoprotein (E. Hartter, H.R., unpublished) one can exclude the futher hypothetical possibility that the presence of an extra peptide in the in vitro product might be compensated on dodecylsulfate gels by lack of a carbohydrate part present in the in vivo product.

In vitro translation of catalase T mRNA can be used in connection with our studies on the regulation of catalase formation. Such experiments are now in progress in our laboratory.

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