The Induction of a Ribosomal Ribonuclease in *Saccharomyces cerevisiae*

Bernd Schulz-Harder * and Claudia Kücherer

Freie Universität Berlin, Fachbereich Biologie WE 3, Institut für Biochemie und Molekularbiologie, Ehrenbergstraße 26–28, D-1000 Berlin 33

Z. Naturforsch. **35 c,** 168–170 (1980);
received February 13/September 5, 1979

Yeast, Ribosomes, Ribonuclease, Cycloheximide, Antimycin

The ribosomal ribonuclease of yeast is induced after a withdrawal of glucose. Cycloheximide inhibits the synthesis of the enzyme under conditions of induction but antimycin shows no effect. Hence, the increase of the ribonuclease activity during growth of yeast is due to newly synthesized enzyme, and the induction does not depend on respiration.

Introduction

Yeast ribonucleases are found in the ribosomal fraction [1] and in the postribosomal supernatant [2]. In contrast to the free ribonuclease activity, the ribosome-bound ribonuclease activity increases during the growth of yeast [3, 4]. Stärk and Jaenicke [5] suggested that the ribosomal ribonuclease is induced by oxygen after consumption of glucose, depending on the intact and coupled respiratory chain in the mitochondria.

In the present report we show that the induction of the ribosomal ribonuclease is actually due to a lack of glucose, and is not dependent on respiration.

Materials and Methods

The tetraploid strain 2200 of *Saccharomyces cerevisiae* [6] was used. The cells were grown in a medium containing 0.5% pepton, 1% Difco yeast extract and 4% glucose at 30 °C, and harvested in the fermentative growth phase.

For the induction of the ribonuclease, the cells were washed with distilled water at 0 °C and then incubated in induction medium consisting of 0.5% pepton, 1% Difco yeast extract and 0.05% glucose at 30 °C for 4 h. Growth was followed by measurement of the turbidity at 525 nm.

Ribosomes were prepared according to the method described by Schulz-Harder and Lochmann [7]. Yeast cells were suspended in buffer A containing 30 mM Tris-HCl, pH 7.5, 200 mM KCl, 5 mM $MgCl_2$, 0.25 mM EDTA, 6 mM 2-mercaptoethanol and 20% (v/v) glycerol. The cell suspension (5 g/20 ml) was poured into a French Pressure Cell (American Instrument) and frozen in an ethanol/dry ice bath. The frozen mixture was then forced through the outlet (inside diameter 1.4 mm) at a pressure of 20000 lb/inch^2. Following the disruption of the cells, the homogenate was centrifuged for 10 min at 18000×*g* in a Sorvall centrifuge. The postmitochondrial supernatant was made 1% in Brij 58 (Serva, Heidelberg, Germany). 20 ml portions of this supernatant were layered on sucrose cushions of 14 ml of 1 M sucrose in buffer A. After centrifugation for 18 h at 131000×*g* in a SW 27 rotor at 2 °C the supernatants were removed by aspiration, the pellets were quickly rinsed with distilled water, and dissolved in buffer A having 50 mM KCl.

Ribosomal RNA (rRNA) was prepared from yeast ribosomes according to the method of Brawerman [8]. The rRNA was denatured by incubation with glyoxal [9] and was characterized by size after electrophoresis on 1.5% agarose gels. As shown in Fig. 1, the rRNA, used as endogenous substrate in the autodegradation assay (see below), was not degraded by the ribonuclease during the preparation

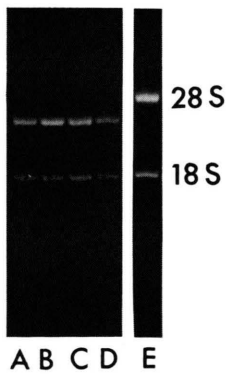

Fig. 1. Electrophoresis of rRNA on 1.5% agarose gels. The RNAs were stained with ethidium bromide and photographed under UV light. Ribosomal RNA from yeast cells: (A) harvested in the fermentative growth phase, (B) after incubation for 4 h in the induction medium, (C) after incubation for 4 h in the induction medium containing 1 mM cycloheximide, (D) after incubation for 4 h in the induction medium containing 2 µg/ml antimycin, (E) 28 S and 18 S rRNA from rat liver used as markers.

* Present address: National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U. K.

Reprint requests to B. Schulz-Harder.
0341-0382/80/0100-0168 $ 01.00/0

of ribosomes or during incubation of yeast cells under different sets of conditions.

In order to determine the ribonuclease activity the autodegradation of ribosomes was assayed in a 0.1 ml of a mixture containing 7 to 11 mg/ml ribosomes, 30 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 6 mM 2-mercaptoethanol and 10 mM EDTA. The incubation was performed at 35 °C. At various times 18 µl samples were removed from the incubation mixture and mixed with 80 µl of 25% perchloric acid and 0.75% uranylacetate. After dilution with 0.9 ml distilled water and centrifugation for 5 min at 5400×*g* (Bio-Dynamics Select-a-Fuge 24) the optical densities of the supernatants were measured against a blank at 260 nm.

$$(1 \text{ unit} = 0.1\ OD_{260\ nm} \times min^{-1} \times ml^{-1}) .$$

Cycloheximide and antimycin were obtained from Serva (Heidelberg, Germany).

Results and Discussion

In accordance with the results of Stärk and Jaenicke [5], we have also found that the decrease of the glucose concentration during growth of yeast is accompanied by an increase in the ribosomal ribonuclease activity (Fig. 2). In order to elucidate whether the shortage of glucose is responsible for the increase of the ribonuclease activity, glycolytic growing yeast cells (medium containing 4% glucose) were transferred to media with various glucose concentrations. Ribosomes derived from cells incubated in a medium containing 0.5% glucose had the highest ribonuclease activity, while cells incubated without glucose possessed ribosomes with low ribonuclease activity, as is indicated in Table I. For further investigations, a medium with 0.05% glucose was used, because no growth of yeast was detectable and the induction of ribonuclease was sufficient.

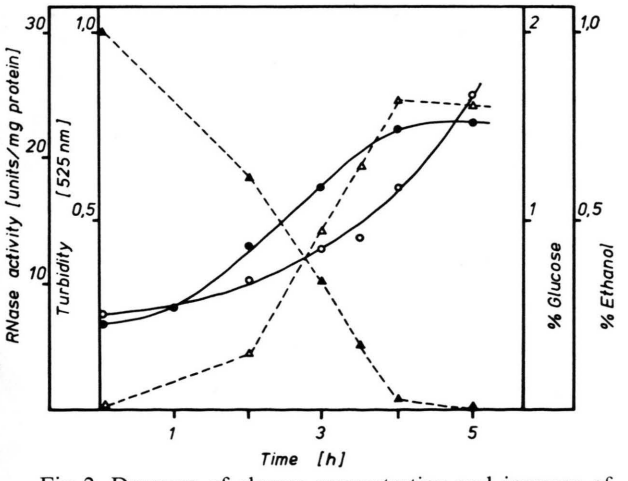

Fig. 2. Decrease of glucose concentration and increase of ethanol concentration and ribosomal ribonuclease activity during growth of yeast. ●—● turbidity (525 nm), ○--○ ribonuclease activity, ▲---▲ glucose concentration, △---△ ethanol concentration. (Medium: 0.5% pepton, 1% Difco yeast extract, 2% glucose.)

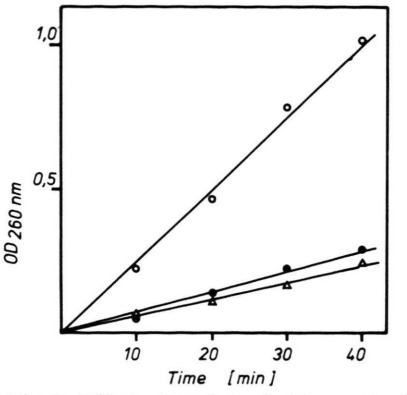

Fig. 3. Effect of cycloheximide on the induction of ribonuclease. Yeast cells were incubated for 4 h in a medium containing 0.5% pepton, 1% Difco yeast extract and 0.05% glucose. ○--○ without cycloheximide, ●--● 1 mM cycloheximide, △--△ control (Yeast cells were not incubated under conditions of induction).

The increase of ribonuclease activity during growth of yeast could be due to an activation of ribonuclease or to newly synthesized enzyme molecules. The complete inhibition of the induction by cycloheximide (Fig. 3) let us infer that the ribonuclease is newly synthesized after the withdrawal of glucose.

Table I. Induction of ribonuclease by reduction of the glucose concentration. Cells were grown in a medium consisting of 0.5% pepton, 1% Difco yeast extract and 4% glucose and then transferred to media with glucose concentrations as indicated. After 4 h of incubation at 30 °C the cells were harvested and ribosomes were prepared. Cells used for the control were not incubated.

Glucose Concentration %	Turbidity (525 nm) 0 h	4 h	Specific Activity units/mg protein
–	0.43	0.45	8.6
0.01	0.46	0.46	11.2
0.05	0.44	0.46	17.2
0.10	0.44	0.64	18.6
0.50	0.45	0.76	20.1
control	–	–	4.4

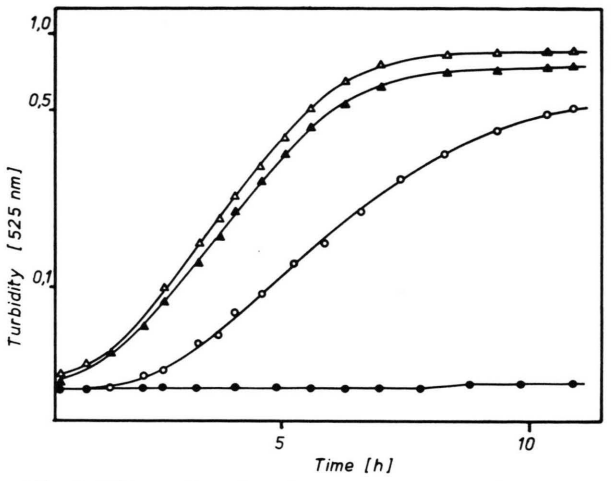

Fig. 4. Effect of antimycin on aerobic growing yeast. ○—○ without antimycin, ●—● 2 µg/ml antimycin. (Medium: 0.5% pepton, 1% Difco yeast extract, 3% glycerol), △—△ without antimycin, ▲—▲ 2 µg/ml antimycin. (Medium: 0.5% pepton, 1% Difco yeast extract, 2% glucose.)

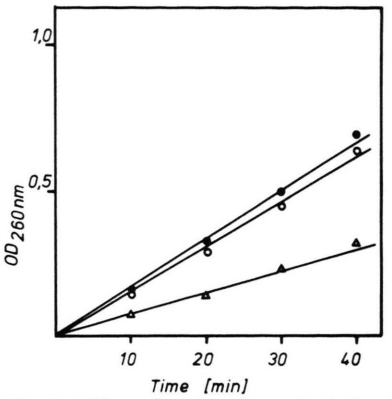

Fig. 5. Effect of antimycin on the induction of ribonuclease. Yeast cells were incubated for 4 h in a medium containing 0.5% pepton, 1% Difco yeast extract and 0.05% glucose. ○—○ without antimycin, ●—● 2 µg/ml antimycin, △—△ control. (Yeast cells were incubated under conditions of induction.)

It is well known that many yeasts show marked impairment of respiratory capability when glucose is abundant in the medium [10]. Cells of *Saccharomyces cerevisiae* change from the glycolytic to the aerobic metabolism when the glucose concentration falls below a critical point [11], and it could be argued that the induction of the ribonuclease is coupled with this change. By inhibition of the induction of ribonuclease with 2,4-dinitrophenol, the synthesis of the enzyme was shown to be connected with the intact and coupled respiratory chain [5]. However, since 2,4-dinitrophenol affects, in addition to the electron transport chain in the mitochondria, the uptake of cytosine [12] and purines [13] as well as the proton gradient at the cell-membrane [14], it is difficult to envisage a direct connection between induction of the ribonuclease and respiration. To analyse this problem more critically, we incubated yeast cells under conditions of incubation with 2 µg/ml antimycin, which inhibits aerobic growing yeast in a medium containing 0.5% pepton, 1% Difco yeast extract and 3% glycerol (Fig. 4). In Fig. 5 it is demonstrated that antimycin does not affect the induction of the ribonuclease. Similar results could be obtained if oligomycin was substituted for antimycin (data not shown). Thus, the synthesis of the ribonuclease during the change of yeast cells from the fermentative to the aerobic growth phase is due to a lack of glucose and does not depend on respiration.

[1] Y. Nakao, S. Y. Lee, H. O. Halvorson, and R. M. Bock, Biochim. Biophys. Acta **151,** 114–125 (1968).
[2] S. Y. Lee, Y. Nakao, and R. M. Bock, Biochim. Biophys. Acta **151,** 126–136 (1968).
[3] J. Danner and R. S. Morgan, Biochim. Biophys. Acta **76,** 652–655 (1963).
[4] G. D. Rock and B. F. Johnson, Can. J. Microbiol. **16,** 187–191 (1970).
[5] G. F. Stärk and L. Jaenicke, Z. Naturforsch. **26 b,** 328–336 (1971).
[6] W. Laskowski, Z. Naturforsch. **17 b,** 93–108 (1962).
[7] B. Schulz-Harder and E.-R. Lochmann, Z. Naturforsch. **31 c,** 169–173 (1976).
[8] G. Brawerman, Methods in Enzymology, **Vol. 30,** Part F, pp. 506–512, Academic Press, New York 1974.
[9] G. K. McMaster and G. G. Carmichael, Proc. Nat. Acad. Sci. USA **74,** 4835–4838 (1977).
[10] A. Sols, C. Gancedo, and G. Dela Fuente, The Yeasts **Vol. 2,** pp. 271–307, Academic Press, New York 1971.
[11] C. Beck and H. K. Meyenburg, J. Bacteriol. **96,** 479–486 (1968).
[12] M. R. Chevallier, R. Jund, and F. Lacroute, J. Bacteriol. **122,** 629–641 (1975).
[13] M. Labischinski, Doctoral Thesis, Freie Universität Berlin 1977.
[14] E. Pavlasova and F. M. Harold, J. Bacteriol. **98,** 198–204 (1969).