Occurrence of "Stress"-Proteins in Yeast after Heat-Shock, Acrylonitrile Treatment and during the Stationary Growth Phase

Ulrich Pfeffer and Bernd Schulz-Harder *

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

Z. Naturforsch. **40 c**, 26–28 (1985); received September 17, 1984

Heat-Shock, Acrylonitrile, Ribonuclease, *Saccharomyces cerevisiae*

The response of yeast cells to different kinds of "stress" is not identical. Cells of the stationary growth phase synthesize three new proteins of molecular weights 68, 27 and 24 kD, compared with cells of the exponential growth phase, while heat-shocked cells exhibit new proteins of 100, 90, 84, 70 and 24 kD. After treatment with acrylonitrile two new proteins with molecular weights of 70 and 46 kD appear. However, all three kinds of "stress" lead to the induction of a ribonuclease.

Introduction

When cells of most organisms are exposed to elevated temperatures, they respond with the induction of a small number of heat-shock proteins [1]. An analogous "stress" response has been observed when chick cells were treated with sulfhydryl oxidants such as sodium arsenite [2], kethoxal bis (thiosemicarbazone), copper complex [3], disulfiram [4], iodoacetamide and *p*-chloromercuribenzoate [5]. Other "stress"-protein inducers are canavanine [6, 7], histidinol [8], cadmium [6] and trauma of mammalian tissues [9]. However, the occurrence of "stress"-proteins can vary between different organisms, and with the kind of "stress" which has been applied. In yeast hyperthermic "stress" or glucose starvation lead to the induction of a ribonuclease which normally appears in the stationary growth phase [10]. In this report we compare the "stress"-proteins of yeast cells derived from the stationary growth phase with heat-shocked and acrylonitrile treated cells, and show that the ribonuclease activity is also enhanced after acrylonitrile treatment.

Materials and Methods

Cells of the diploid strain R XII of S. cerevisiae (a kind gift of Dr. A. Kotyk, Prague) were grown in a medium containing 0.5% peptone, 1% Difco yeast extract and 4% glucose at 30 °C, and harvested in the fermentative growth phase. The cells were transferred to the same fresh medium and incubated at 39 °C (for heat-shock) or at 30 °C in the presence of 0.05% acrylonitrile.

To label proteins 3.2×10^7 cells were incubated with 10 µCi of a [^{14}C]amino acid mixture (NEN) for 15 min. Growth was stopped by addition of NaN_3 (20 mM) and cycloheximide (200 µg/ml), and the cell suspension was kept on ice for 5 min. After centrifugation and washing with 2 mM NaN_3 the cells were frozen at −20 °C. The frozen samples were mixed with 200 µl of a solution consisting of 50 mM Tris-HCl, pH 6.8, 10 mM 2-mercaptoethanol, 2% SDS, 10% glycerol, 0.1% bromophenolblue, and heated for 5 min at 90 °C. Then the cells were homogenized by vortexing with 200 µl glass beads (diameter: 0.45–0.55 mm) for 1 min. After pelleting the glass beads and cell debris the supernatants were heated again for 90 s, and samples containing equal amounts of radioactivity were applied on a SDS-polyacrylamide gel [11].

Fluography of the gels was performed according to the method of Laskey and Mills [12]. The activity of a ribosome-associated ribonuclease [13] was assayed as described earlier [10]. (Ribonuclease activity: 1 unit = 0.1 $A_{260\,nm/min/ml}$.)

Results and Discussion

We have investigated the reaction of yeast cells to different kinds of "stress" by analyzing the protein pattern of fluorograms of SDS-polyacrylamide gels with a laser densitometer. Fig. 1 shows the protein profiles of cells of the exponential growth phase, the stationary growth phase and cells grown at 39 °C

* Present address: Dr. B. Schulz-Harder, RWTH Aachen, Anatomie I, Melatener Straße 211, D-5100 Aachen.

0341-0382/85/0100-0026 $ 01.30/0

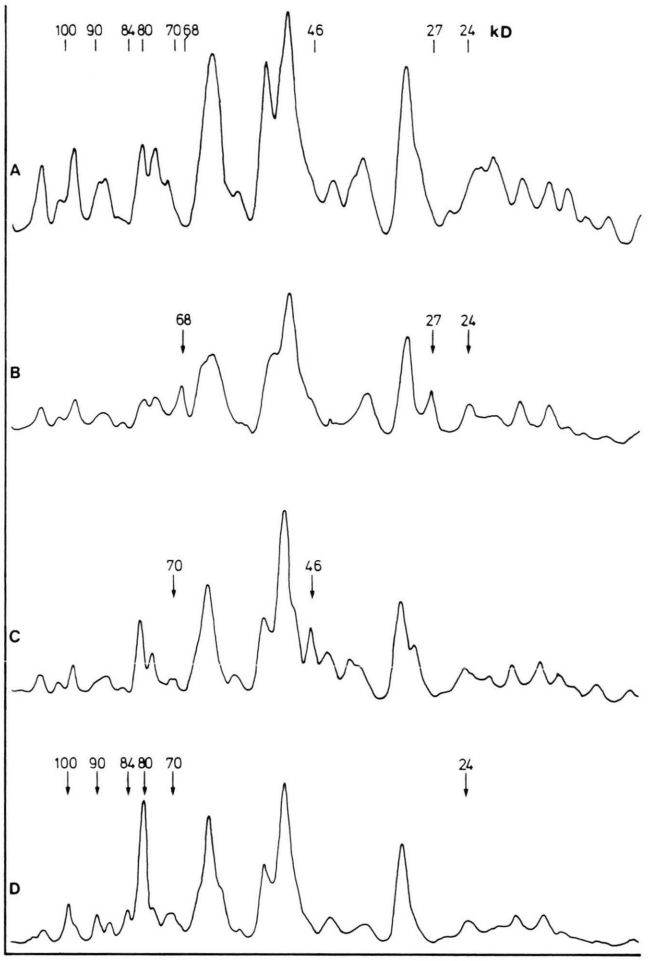

Fig. 1. Analysis of ^{14}C-labelled whole-cell proteins by SDS-polyacrylamide gel electrophoresis of (A) exponential growth phase cells grown at 30 °C, (B) stationary growth phase cells, (C) acrylonitrile treated cells (exponential growth phase cells incubated at 30 °C for 1 h in the presence of 0.05% (v/v) acrylonitrile), and (D) heat-shocked cells (exponential growth phase cells incubated at 39 °C for 1 h). The cells were labelled with ^{14}C-amino acids as described in Materials and Methods. The fluorographs were scanned by an LKB 2202 Ultrascan laser densitometer. Molecular weights of proteins were determined by comparison with M_r markers: rabbit myosin (205 000), *E. coli* β-galactosidase (11 600), rabbit phosphorylase B (97 000), bovine serum albumin (66 000), ovalbumin (45 000) and carbonic anhydrase (29 000).

or in the presence of 0.05% acrylonitrile. It is obvious that under "stress" conditions some proteins are newly synthesized or their synthesis has been enhanced while the synthesis of others decreased. However, the response of yeast cells to different kinds of "stress" is not identical. Cells of the stationary growth phase, which suffer a lack nutrition, exhibit new proteins of 68, 27 and 24 kD, while cells treated with acrylonitrile have predominant protein peaks of 70 and 46 kD. The most dramatic change in the protein pattern occurs after heat-shock. Six new protein bands with molecular weights of 100, 90, 84, 70, and 24 kD appear or are enhanced.

Because the change of protein synthesis after heat-shock in yeast cells is due to regulatory events on the transcriptional level and increased degradation of mRNA in the cytoplasm [14], we expected alterations of the mRNA populations after "stress" treatment. In order to visualize these changes we made crosshybridization experiments with the Northern-hybridizing technique. The cDNA was prepared from mRNA of control and "stress"-treated cells. Despite differences between the protein profiles, there were no alterations detectable with the hybridization technique (results not shown). Because the hybridization of cDNA to mRNA is a very sensitive method, we presume that this result is due to a high background of mRNAs which are transcribed under both normal and "stress" conditions.

The ribosomal content of yeast cells of the stationary growth phase [15] as well as of the cells grown at elevated temperatures [16] is lower than in

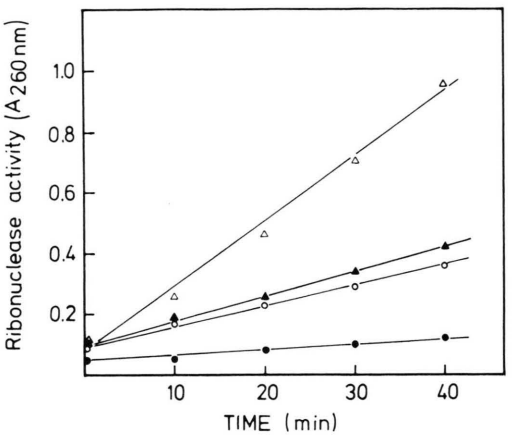

Fig. 2. Ribonuclease activities of exponential growth phase cells (●—●), stationary growth phase cells (○—○), acrylonitrile treated cells (exponential growth phase cells incubated at 30 °C for 3 h in the presence of 0.05% (v/v) acrylonitrile) (▲—▲), heat-shocked cells (exponential growth phase cells incubated at 39 °C for 3 h) (△—△). The ribonuclease activity was assayed by autodegradation of ribosomes in 0.1 ml of a mixture containing 5 mg ribosomes/ml, 30 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 6 mM 2-mercaptoethanol and 20 mM EDTA at 35 °C. The reaction was stopped at the times indicated by mixing 18 µl of the mixture with 80 µl of 25% perchloric acid and 0.75% uranyl acetate. After dilution with 1 ml distilled water and centrifugation the absorbances of the supernatants were measured against a blank at 260 nm.

cells of the exponential growth phase cultivated at 30 °C. In previous publications we have reported on the induction of a ribosome-associated ribonuclease during the stationary growth phase [17], and after hyperthermic shock [10], and suggested that the decrease of the RNA content is due to the occurrence of this enzyme. Recently, Lochmann *et al.* [18] have found that increasing acrylonitrile concentrations lead to a reduction and finally to a total inhibition of the rate of RNA and ribosome synthesis. In parallel the content of free and membrane-bound ribosomes is diminished. Because the decrease of the ribosome content is greater than would be expected from the reduced rate, we assumed a degradation of the ribosomes. In Fig. 2 it is demonstrated that acrylonitrile induces a ribonuclease to the same level as it is present during the stationary growth phase, but the induction by heat-shock is approximately three times higher than due to acrylonitrile treatment (12.0 units and 4.1 units ribonuclease activity, respectively). A comparison of the reactions of yeast cells to different "stress" inducers shows that heat-shock triggers the strongest response. However, in yeast the induction of a ribonuclease seems to be a common response to "stress".

[1] Heat Shock: From Bacteria to Man (M. J. Schlesinger, M. Ashburner, and A. Tissières, ed.), Cold Spring Harbor, New York 1982.
[2] D. Johnston, H. Oppermann, J. Jackson, and W. Levinson, J. Biol. Chem. **255,** 6975–6980 (1980).
[3] W. Levinson, H. Oppermann, and J. Jackson, Biochim. Biophys. Acta **518,** 401–412 (1978).
[4] W. Levinson, P. Mikelens, H. Oppermann, and J. Jackson, Biochim. Biophys. Acta **519,** 65–75 (1978).
[5] W. Levinson, H. Oppermann, and J. Jackson, Biochim. Biophys. Acta **606,** 170–178 (1980).
[6] L. E. Hightower and F. P. White, J. Cell Physiol. **108,** 261–275 (1981).
[7] P. M. Kelley and M. J. Schlesinger, Cell **15,** 1277–1286 (1978).
[8] A. Ron and D. N. Wheatley, Exp. Cell Res. **153,** 158–166 (1984).
[9] F. P. White, J. Neurosci. **1,** 1312–1319 (1981).
[10] B. Schulz-Harder, FEMS Microbiology Letters **17,** 23–26 (1983).
[11] U. K. Laemmli, Nature **227,** 680–685 (1970).
[12] R. A. Laskey and A. D. Mills, Eur. J. Biochem. **56,** 335–341 (1975).
[13] B. Schulz-Harder, U. Swida, and N. Käufer, Biochim. Biophys. Acta **465,** 173–182 (1979).
[14] S. Lindquist, Nature **293,** 311–314 (1981).
[15] G. D. Rock and B. F. Johnson, Can. J. Microbiol. **16,** 187–191 (1970).
[16] J. Wessel and E. R. Lochmann, Z. Naturforsch. **35c,** 171–172 (1980).
[17] U. Swida, B. Schulz-Harder, C. Kücherer, and N. Käufer, Biochim. Biophys. Acta **652,** 129–138 (1981).
[18] E. R. Lochmann, W. Ehrlich, and M. Mangir, Ecotoxicol. Environ. Safety **8,** 162–166 (1984).