RNA SYNTHESIS OF YEAST IN THE PRESENCE OF CYCLOHEXIMIDE.

H. Fukuhara

(Laboratoire de Génétique physiologique du C.N.R.S., Gif-sur-Yvette, Seine-et-Oise, France.)

Received November 28, 1964

Cycloheximide (Acti-dione) is an antibiotic produced by Streptomyces griseus (Whiffen et al., 1946). This compound inhibits growth of many different species of yeast. Various laboratories have studied the effect of this inhibitor on yeast metabolism (Kerridge, 1958; Latuasan and Berends, 1958; Tsukada et al., 1962; Wescott, 1962). Siegel and Sisler (1964) have recently shown in the course of their in vitro experiments that cycloheximide inhibits protein synthesis, probably at the level of the transfer of activated amino acids onto the ribosomes. The present paper describes the study of the in vivo effect of this inhibitor on the synthesis of RNA and protein, during induction of respiratory enzymes in yeast (occurring without growth, in the absence of an added nitrogen source). We found that at certain low concentrations, cycloheximide totally inhibits protein synthesis, whereas RNA synthesis is only slightly depressed. The main object of this work is to characterize the RNA which is being formed under these conditions where protein synthesis is inhibited.

We have previously shown (Shortman and Fukuhara, 1963) that a special class of RNA is synthesized during induction of respiratory enzymes in non-growing cells. The molecular size and the base ratios of the RNA species are different from those of ribosomal and transfer RNA. This class of RNA can be detected by its extremely high turnover rate, in the course of radioactive short pulse experiments. After a prolonged pulse, all the incorporated label is recovered in ribosomal and transfer RNA. This fact was interpreted kinetically as a transformation of high turnover RNA into stable RNA. The rate of this transformation is higher in oxygen-induced than non-induced yeast. The use of cycloheximide allows us to characterize the RNA synthesized in the presence of inducer but without enzyme synthesis.

The yeast (Saccharomyces cerevisiae, strain Yeast Foam) was grown in strict anaerobiosis in natural medium, then suspended in glucose-buffer. The yeast suspension was aerated at 25°. Oxygen induces the synthesis of a full complement of respiratory enzymes

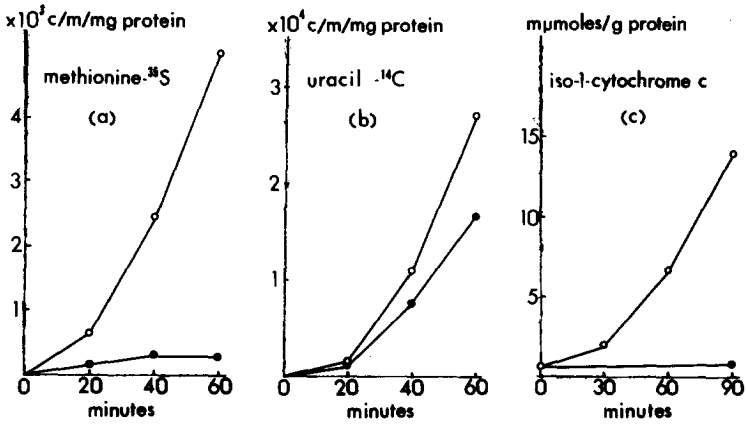

Fig. 1: Effect of cycloheximide on incorporation of a) methionine-^{35}S and b) uracil-^{14}C, and c) on induction of iso-1-cytochrome c. Anaerobically grown cells were aerated at 25° in succinate buffer (0.05 M, pH 4.2), containing 5% glucose, with or without cycloheximide (25 μg /ml). DL-methionine-^{35}S (1.3 μC/μmole, 0.1 mM) or uracil-^{14}C (10 μC /μmole, 0.I mM) was added at the beginning of aeration. Samples taken at various moments of incubation were precipitated with 5% trichloracetic acid, and filtered on Millipore membranes for the measurement of radioactivity. For the determination of the quantities of iso-1-cytochrome c, a parallel bigger scale incubation was run; cytochrome c was measured according to Sels et al.(in press).
o——o : control; ●——● : with cycloheximide.

(Slonimski, 1956). During the first 60 min of induction, uracil-^{14}C or methionine-^{35}S was added to the suspension, in the presence or in the absence of cycloheximide (25 μg/ml). Samples taken at definite intervals during incubation were precipitated by trichloracetic acid. The rate of incorporation of labelled methionine into the precipitable material was reduced by 95% by cycloheximide (Fig. 1 a). The synthesis of iso-1-cytochrome c (Slonimski et al., 1963) was equally inhibited by nearly 100% (Fig. 1 c). In contrast, the rate of uracil incorporation was reduced by 30% (Fig. 1 b). After 6o min incubation with the radioactive precursors, the yeast cells were washed and broken in a Nossal shaker. The supernatant of 20 min centrifugation at 24.000xg was centrifuged again at I60.000xg for 4 hours and the pellet was collected. One part of this pellet was analyzed in a sucrose gradient according to current centrifugation technique (Roberts et al., 1958). Absorbancy measurements at 260 mμ revealed that the ribosomes were in one peak. Newly synthesized RNA and proteins were detected by the radioactivity counts. In the control system (without cycloheximide) the ribosomal peak was completely labelled with uracil-^{14}C and methionine-^{35}S, showing that de novo synthesis of ribosomes occurred during in-

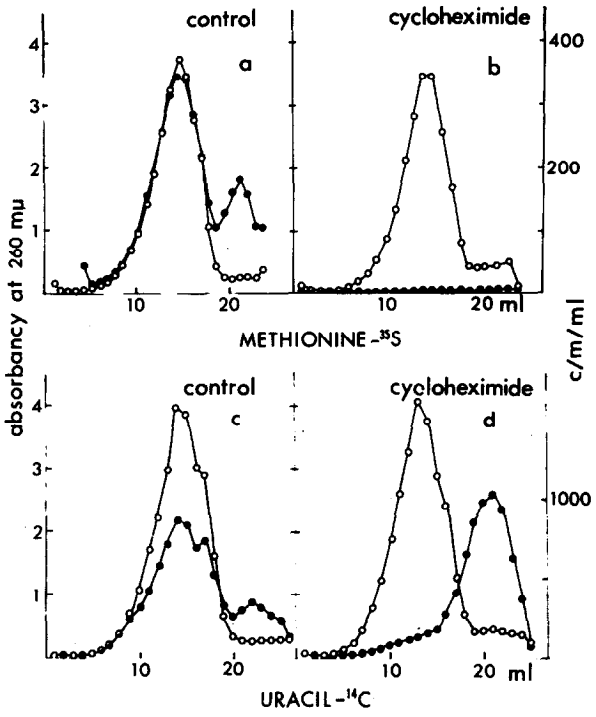

Fig. 2: Effect of cycloheximide on the synthesis of ribosomes. Anaerobically grown yeast was induced as in Fig. 1. After incorporation of methionine-^{35}S (a and b) or uracil-^{14}C (c and d) for the first hour of aeration, cells were washed in cold buffer (Tris-HCl 0.02 M, pH 7.4, Mg acetate $1 \cdot 10^{-5}$M) and broken in a Nossal shaker. After removal of large particles by centrifugation at 24.000xg for 20 min, the supernatant was centrifuged at 160.000xg for 4 hours. The pellet was dialyzed against the buffer. An aliquot was layered on a sucrose gradient (4 - 15%, in buffer, 25 ml). After centrifugation at 56.000xg for 4 hours, the content of the tube was collected from the bottom in 1 ml fractions. On each fraction, 260 mμ absorbancy was measured after suitable dilution. Radioactivity was measured after precipitation with 5% trichloracetic acid in presence of 500 μg of serumalbumine. The main UV absorbing peak in each figure corresponds to a mixture of 60 S and 40 S ribosomes. The minor radioactive peak in a) represents radioactive free proteins. o—o : absorption at 260 mμ; ●—● : radioactivity.

---

duction (Fig. 2 a and c). On the contrary, in the presence of the inhibitor, there was no incorporation of labelled uracil or methionine into ribosomes (Fig. 2 b and d): incorporation of labelled methionine was practically zero, and labelled uracil was incorporated into material sedimenting much more slowly (less than 20 S) than ribosomes (Fig. 2 d). This slower sedimenting material was precipitable by trichloracetic acid and was rendered non precipitable by ribonuclease

treatment. The uracil-labelled material formed in the presence of the inhibitor was therefore a species of RNA, without any newly formed protein associated to it, since there was no radioactive methionine incorporated into this fraction.

Starting with the same uracil-labelled yeast, total RNA was extracted by phenol plus Duponol. The labelled RNA was analyzed by sedimentation in a sucrose gradient: in the control system without inhibitor, the newly formed RNA was entirely of ribosomal (24 S and 16 S) and transfer (4 S) type; however, in the presence of cycloheximide,

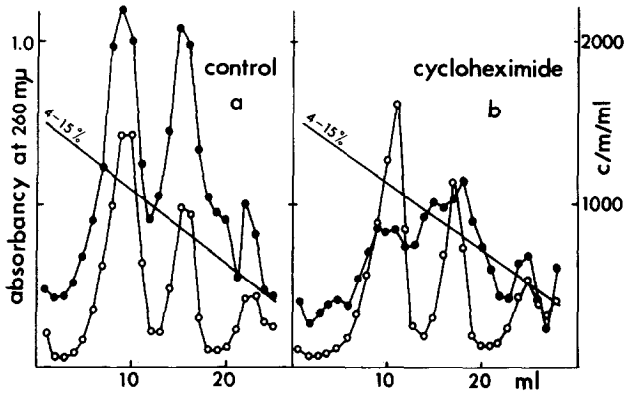

Fig. 3: Sedimentation of RNA synthesized in presence of cycloheximide. Yeast cells were labelled with uracil-^{14}C as in Fig. 2. RNA was extracted and purified according to Shortman and Fukuhara (1963). An aliquot of RNA solution was layered on a sucrose gradient (4 - 15%, in 0.1 M NaCl) and centrifuged for 14 hours at 56.000xg. The content of the tube was analyzed as in Fig. 2. o——o : absorption at 260 mμ; ●——● : radioactivity. The two major UV absorbing peaks represent ribosomal 24S and 16S RNA, and the minor peak , 4S RNA.

---

the labelled RNA was a mixture of various types of RNA, the major part of which had a size corresponding to about 16 S (Fig. 3 a and b). It should be noted that this sedimentation profile of labelled RNA is very much like that of high turnover RNA detected when a short pulse (2-5 min) of radioactive uracil had been applied under the same conditions (see Fig. 6 a and b in Shortman and Fukuhara, 1963). In the noted case, the initially labelled RNA rapidly changed during extended incubation into ribosomal and transfer RNA; whereas in the presence of cycloheximide, this fraction of RNA (short pulsed) remained unchanged even after 60 min. Further studies are designed to determine the site of accumulation of the RNA in the cell.

Formation of this type of RNA in the presence of cycloheximide occurred not only in this particular system in the absence of growth, but also in aerobic yeast cultures, exponentially growing on natural glucose medium, with added cycloheximide (20 µg/ml) and labelled uracil: during the first hour after addition of the inhibitor, the growth rate was depressed by 60%, and at the same time, labelled RNA accumulated in the same manner, as seen in Fig. 2 b.

It is therefore concluded that, during inhibition of protein synthesis by cycloheximide, there is an accumulation of high turnover RNA accompanied by other cytoplasmic RNA species.

Acknowledgements: We are grateful to Dr. P. Slonimski for his continuous interest in this work. The technical assistance of Miss C. Genin was greatly appreciated. This work received financial support from the Délégation Générale à la Recherche Scientifique et Technique, and from the Commissariat à l'Energie Atomique.

REFERENCES.

Kerridge, D., (1958) J.Gen.Microbiol., 19, 497.
Latuasan, H.E. and Berends, W., (1958) Rec.Trav.chim., 77, 416.
Roberts, R.B., Britten, R.J. and Bolton, E.T., (1958) in R.B. Roberts, Microsomal Particles and Protein Synthesis, Pergamon Press, p.85.
Sels, A., Fukuhara, H., Péré, G. and Slonimski, P., (1965) Biochim. Biophys.Acta, in press.
Shortman, K., and Fukuhara, H., (1963) Biochim.Biophys.Acta, 76, 501.
Siegel, M.R., and Sisler, H.D., (1964) Biochim.Biophys.Acta, 87,70, 83.
Slonimski, P., (1956) 3rd Internat.Congr.Biochem., Brussels. p. 242.
Slonimski, P., Acher, R., Péré, G., Sels, A., and Somlo, M., (1963) Internat.Symp. on Mechanisms of Regulation of Cellular Activities in Microorganisms, Marseille, Gordon and Breach, New York, in press.
Tsukada, Y., Sugimori, T., Imai, K., and Katagiri, H., (1962) J. Bacteriol., 83, 70.
Wescott, E.W., (1962) Thesis, University of Maryland, p.27.
Whiffen, A.J., Bohonas, J.N., and Emerson, R.L., (1946) J. Bacteriol. 52, 610.