ACCUMULATION OF RNA WITH A DNA LIKE BASE COMPOSITION IN SACCHAROMYCES CARLSBERGENSIS IN THE PRESENCE OF CYCLOHEXIMIDE

S.R. de Kloet

Philips Research Laboratories
Philips' Gloeilampenfabrieken
Eindhoven - Netherlands

Received March 30, 1965

Cycloheximide has been shown to be a powerful inhibitor of protein biosynthesis in yeast and numerous other organisms but not in bacteria (Siegel et al. 1964). The effect of the antibiotic on RNA formation has been studied by the same authors and by Kerridge (1958). Siegel et al. observed a stimulation of RNA synthesis in Saccharomyces pastorianus by cycloheximide, the base composition of the RNA formed in the presence of the antibiotic was found to resemble the composition of yeast ribosomal RNA. Kerridge studying Saccharomyces carlsbergensis found an inhibition of RNA synthesis caused by the drug. Recently Fukuhara (1965) reported the accumulation of RNA with abnormal sedimentation properties during the incubation of Saccharomyces cerevisiae with cycloheximide. The present paper describes some of the results of studies on the effect of the antibiotic on RNA synthesis in Saccharomyces carlsbergensis. The data indicate that there occurs an accumulation of RNA with a more DNA like base composition and sedimentation properties strongly differing from ribosomal RNA during incubation of cells or protoplasts of this organism with cycloheximide.

MATERIALS AND METHODS

Saccharomyces carlsbergensis (str. 74 N.C.Y.C.) was grown as described earlier (de Kloet, 1961). Protoplasts were prepared as described in the same paper. High molecular weight RNA labeled with C^{14} uracil was isolated from protoplasts by treatment with sodium lauryl sulphate and phenol (de Kloet, to be publ.). Sucrose density gradient centrifugation was

carried out employing linear 20 to 5 per cent gradients containing 0.1 M NaCl, 0.002 M versene and 0.02 M Na-acetate pH 5.2. One ml samples of the RNA were applied on top of the 29 ml gradient and centrifuged at 2°C for 16 hrs at 23000 rpm in the SW 25 rotor of the Spinco preparative ultracentrifuge model L. Fractions were collected after puncturing the bottom of the tube. Radioactivity was estimated after the addition of 2 mg protoplast protein as a carrier followed by precipitation and washing with trichloroacetic acid, alcohol containing 0.1 M potassium acetate, alcohol and acetone. The optical density at 260 mμ of the fractions was estimated after thirty fold dilution.

For the extraction of P^{32} labeled RNA for estimation of the base composition, yeast cells were previously treated with phenol to remove soluble RNA (Monier et al., 1960) and subsequently washed with trichloroacetic acid, alcohol-potassium acetate, alcohol, chloroform-ether-alcohol mixture and acetone. RNA was extracted by two successive treatments with 5 ml 10 per cent NaCl at 100°C for one hour. The RNA thus obtained was further deproteinized with phenol and finally collected by precipitation with alcohol.

Hydrolysis of the RNA was carried out in 0.5 N KOH for 18 hrs at 37°C. After neutralization with perchloric acid the 2'3' nucleotides were separated on Dowex-1-formate in a formic acid system, according to Osawa et al. (1958). The base composition of the labeled RNA was calculated from the total norit adsorbable counts of the separate nucleotide fractions.

Cycloheximide was obtained from Light & Co (Colnbrook, England).

RESULTS

When yeast protoplasts are incubated for a short time with C^{14}-labeled uracil, the label becomes incorporated into rapidly sedimenting heterogeneous RNA (fig. 1a). Similar phenomena have been observed in a number of other systems like HeLa cells etc. (cf. Scherrer et al., 1962). The addition of cycloheximide does not yield a different sedimentation pattern of the C^{14}-labeled RNA. However, when yeast protoplasts are incubated with C^{14}-labeled uracil for five minutes, followed by a chase with unlabeled uracil for sixty minutes, the presence of cy-

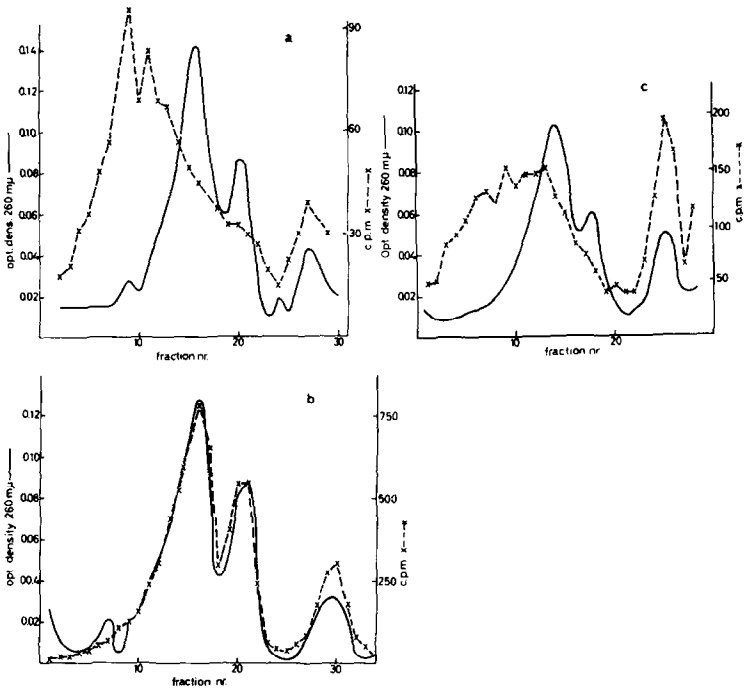

Fig. 1 The effect of cycloheximide on the sedimentation pattern of yeast RNA. Protoplasts (E at 500 mμ = 1) were incubated at 30°C in 25 ml of a medium containing per ml: 120 mg mannitol, 10 mg glucose, 10 mg casamino acids (Difco vitamin free), 20 μmoles K-Na-PO_4 buffer pH 6.3 and 2 μmoles $MgCl_2$. After preincubation with or without cycloheximide (10 μg per ml), 14 μg C^{14}-labeled uracil was added, followed for chase experiments after five minutes by 1 ml 0.05 M unlabeled uracil. (spec. act. C^{14}-labeled uracil = 40 mC per mmole). The incubation was then continued for another sixty minutes. The reaction was terminated by rapid cooling of the vessels containing the protoplast suspension, followed by centrifugation for five minutes in the cold. RNA was isolated and subjected to sucrose density gradient centrifugation as described earlier.
a = five minutes with C^{14}-labeled uracil in the presence or absence of cycloheximide
b = chase experiment in the absence of cycloheximide
c = chase experiment in the presence of cycloheximide

cloheximide in the medium results in a different sedimentation behaviour compared with the normal case where no cycloheximide was added. In the presence of the antibiotic the labeled RNA sediments as a broad peak with its highest activity sedimenting in front of the heaviest ribosomal RNA component, yielding a pattern closely resembling that of the pulse la-

beled RNA together with a peak of material of lower molecular weight.

Estimation of the base composition of the RNA formed in the presence or absence of cycloheximide also revealed differences (Table I). It is clear from the data in table I that the addition of cycloheximide to the incubation medium leads to the accumulation of RNA with a more DNA like base composition as found normally in yeast cells. Essentially the same results were obtained when the base composition of P^{32}-labeled high molecular weight RNA obtained by sucrose density gradient centrifugation was estimated.

TABLE I. The effect of cycloheximide on the base composition of newly synthesized yeast RNA.

	cyt	ad	gu	ur(thy)
- cycloheximide after 40'	19.7	26.0	27.6	26.6
- " " 90'	19.7	26.4	28.2	26.6
+ " " 90'	19.6	30.6	21.8	28.0
yeast RNA	20.7	26.2	27.2	25.5
yeast DNA	18	32	18	32

Yeast cells were incubated in the same medium as described under fig. 1, except for the omission of mannitol. The phosphate concentration was reduced to 0.2 umoles per ml. 0.25 mC P^{32}-labeled orthophosphate was added instead of C^{14}-labeled uracil. Incubation was carried out for 40 or 90 minutes. The isolation of the RNA was carried out as described before. Total yeast RNA base composition was estimated by ultraviolet spectrophotometry of phenol purified yeast RNA isolated from protoplasts. The base composition of yeast DNA was obtained from the literature (Zamenhof, 1950). Values are expressed in moles per cent.

DISCUSSION

The results presented above show that cycloheximide interferes strongly with the normal RNA metabolism in yeast. In experiments to be described in a later paper it has been found that under the circumstances employed here cycloheximide causes a 60 to 90 per cent inhibition of C^{14}-labeled uracil incorporation. It seems as if cycloheximide inhibits specifically the formation of ribosomal RNA and causes the accumulation of messenger RNA. These results differ from those obtained for

chloramphenicol and E. coli, where the RNA synthesized in the presence of the antibiotic has been shown to possess the base composition (Pardee et al., 1957) and molecular properties of ribosomal RNA (Kurland et al., 1962). Little is known about the mechanism by which the cell regulates the formation of its different RNA constituents and it should be remarked that although it seems as if cycloheximide inhibits specifically the formation of ribosomal RNA in Saccharomyces carlsbergensis the possibility cannot be excluded that the antibiotic acts indirectly by preventing the synthesis of ribosomal protein, leaving the newly synthesized ribosomal RNA unprotected and possibly exposed to enzymatic degradation. The present results disagree with those obtained by Siegel et al. (1964) on the base composition of RNA formed in Saccharomyces pastorianus in the presence of cycloheximide. The formation of RNA with an abnormal sedimentation behaviour in the presence of cycloheximide has been shown to occur in Neurospora crassa by Fiala et al. (1965) and in Saccharomyces cerevisiae by Fukuhara et al. (1965). The differences between the results of Fukuhara and those presented here are probably caused by differences in the methods employed for the isolation of the RNA.

The expert technical assistance of Mr. B. Andrean is gratefully acknowledged.

REFERENCES

Fiala, E., F.F. Davis, Biochem. Biophys. Res. Comm. 18, 115 (1965)
Fukuhara, H., Biochem. Biophys. Res. Comm. 18, 153 (1965)
Kerridge, D., J. Gen. Microbiol. 19, 497 (1958)
de Kloet, S.R., Thesis (Utrecht), 1961
Kurland, C.G., M. Nomura, J.D. Watson, J. Mol. Biol. 4, 388 (1962)
Monier, R., M.L. Stephenson, P.C. Zamecnik, Biochim. et Biophys. Acta 43, 1 (1960)
Osawa, S., K. Takata, Y. Hotta, Biochim. et Biophys. Acta 28, 271 (1958)
Pardee, A.B., K. Paegin, L.S. Prestidge, Biochim. et Biophys. Acta 24, 215 (1957)
Scherrer, K., H. Latham, J.E. Darnell, Proc. Natl. Acad. Sci. 49, 240 (1963)
Siegel, M.R., H.D. Sisler, Biochim. et Biophys. Acta 87, 70 (1963)
Zamenhof, S., E. Chargaff, J. Biol. Chem. 187, 1 (1950)