EFFECTS OF CYCLOHEXIMIDE ON RIBOSOMAL RNA SYNTHESIS IN YEAST

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SUMMARY

Yeast cells accumulate a high molecular weight RNA fraction in the presence of the drug, cycloheximide. This fraction appears to have the same properties as the 45S ribosomal precursor in the higher eukaryotes. Thus yeast, although a primitive eukaryote, seems to possess the same mechanism for ribosomal RNA processing as higher organisms.

The mechanism of ribosomal RNA synthesis is now well delineated in both animal cells and bacteria. (E.g. Maden, 1968; Osawa, 1968). The two systems are dissimilar in that the former produces the two dominant species of ribosomal RNA through the processing of a series of precursor molecules; the latter apparently produces ribosomal RNA directly.

We have studied ribosomal RNA synthesis in the fission yeast, Schizosaccharomyces pombe, which in certain aspects resembles both animal cells and bacteria. The yeast cell is eukaryotic, with a bilaminar nuclear membrane containing typical "nuclear pores". The nucleolus resembles that of animal cells, although it is more diffuse in structure (unpublished observations). On the other hand, this yeast cell is bacteria-like in that it has a short generation time, a high content of ribosomes (MacLean, 1965), and no apparent cytoplasmic membrane system or mitotic apparatus (Robinow and Marak, 1966). With such a curious cytological profile, we were interested to know its mode of ribosomal RNA production.

When we analyzed the RNA produced after cycloheximide (Cx) treatment we found that a high molecular weight RNA accumulates. This RNA fraction has characteristics similar to the high molecular weight ribosomal RNA precursor fraction found in other eukaryotes.

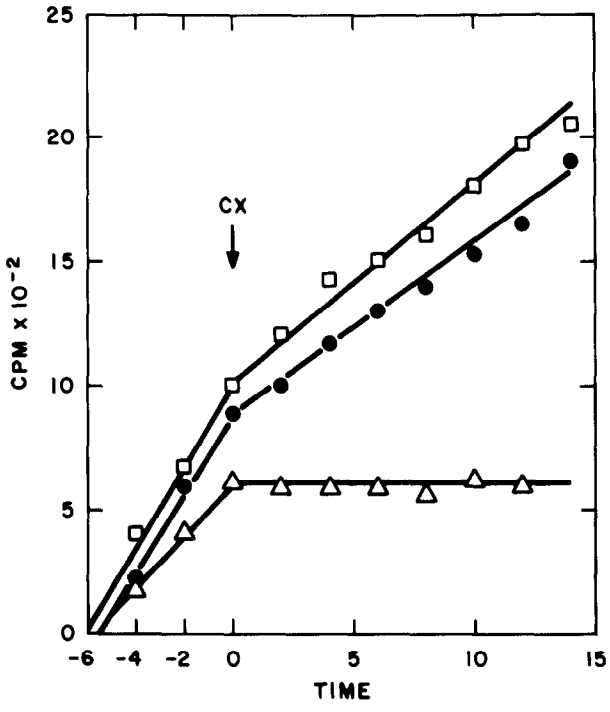

Fig. 1. Effect of cycloheximide on uracil, methionine and lysine, 100 ug cycloheximide (acti-dione, The Upjohn Company) per ml of culture was added 6 minutes after appropriate isotopically labelled compound. 2 ml aliquots were taken at the time indicated into 10% ice cold TCA. The cells were collected on Millipore filters (HAWP) washed with 5% TCA, H_2O, and alcohol, dried and counted as described in the text. □—□, C^{14} uracil; ○—○, H^3 methionine; △—△, H^3 lysine. Uracil and methionine labelling were done in a single experiment, lysine in a separate cell culture under identical conditions.

Fig. 1 shows the incorporation of uracil, lysine, and H^3 labelled methyl of methionine into the yeast following the addition of Cx (Acti-dione, UpJohn) to a final concentration of 100 ug/ml. It can be seen that lysine incorporation into TCA precipitable material stops within one minute, indicating the immediate and complete cessation of protein synthesis. Uracil and methionine counts continue to be incorporated linearly, though at a reduced rate, for over twenty minutes. This concentration of Cx (100 ug/ml) although causing the effects on amino acid and uracil incorporation as shown, is completely reversible, even after 1 hour

exposure. The culture recovers the normal growth rate with 100% viability within 90 minutes following removal (Vincent and Coetzee, unpublished). These results show that RNA synthesis continues in the presence of Cx, and the fact that the methyl group continues to be incorporated at the same rate as uracil, in the absence of protein synthesis, indicates that methylation of the RNA is occurring as described by Wagner, et al (1967) for HeLa cells.

In order to determine the nature of the RNA synthesized following Cx addition, H^3 uracil was added to an exponentially growing culture (O.D. of 0.2) one minute after Cx addition. The cells were then allowed to incorporate

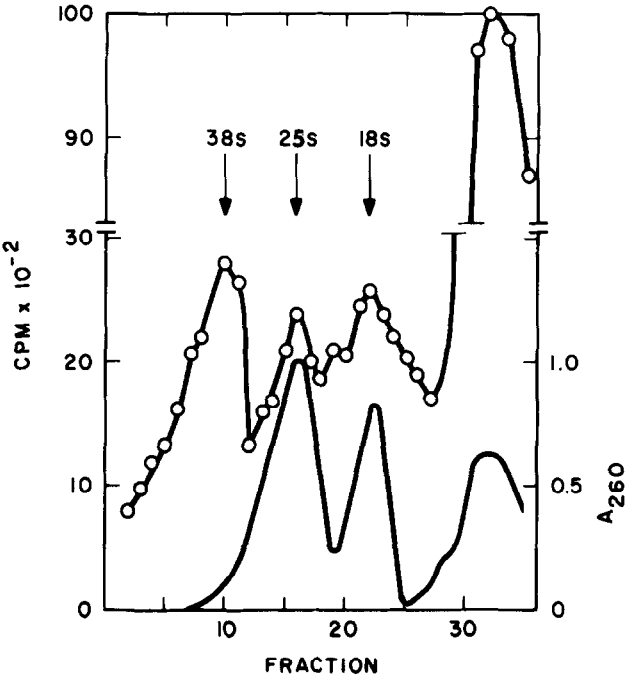

Fig. 2. *Velocity sedimentation of RNA following 15 minutes exposure* to 100 ug/ml cycloheximide. The RNA was dissolved in 0.01M Tris, pH 7.4 with 0.01 M NaCl and 0.5% SDS and layered over a 15-30% sucrose gradient made in 0.01 M NaCl, 0.5% SDS and 10^{-2}M EDTA. The RNA was sedimented for 16 hours at 24k in an SW 25 rotor at 23 . Approximately 0.8 ml fractions were collected with a fraction collector and A_{260} of each fraction was measured. Each fraction was precipitated with cold TCA to 5% final concentration. The ppt were collected on Millipore filters (HAWP), washed with TCA, H_2O and 95% ethyl alcohol, dried and counted in toluene-PPO-POPOP counting fluid in a Beckman DPM-100. All counting was carried out to 2% reproducibility with appropriate corrections for spillover. ——— , A_{260} nm; O———O, H^3 uracil.

for 15 minutes, at which time they were collected, Macaloid added at concentration of 2.5 mg/ml and rapidly frozen (-60 C). The coating of the cells with Macaloid prior to breakage was found to be necessary to produce the > 28S RNA on the gradients. (This was also found by Retel and Planta (1967) in S. carlsbergensis). The cells were suspended in buffer (0.05 M Tris, pH 7.4; 0.005 M $MgCl_2$; 0.025 M KCl and broken at dry ice temperatures in an Eaton press (1962). The pressate was extracted for twenty minutes at 30-35 C with phenol and the aqueous phase precipitated with ethanol.

Gradient analysis of this experiment is shown in Fig. 2. There is a large peak of radioactivity in the 38S region of extremely high specific activity with counts in both the 18 and 25S peaks, assuming linearity of sedimentation. There is also increased synthesis of RNA sedimenting in the 4S region. According to Loening, the "25S" and "18S" fractions in S. pombe have molecular weights of 1.3×10^6 and 0.7×10^6 daltons, respectively, as judged by gel electrophoretic mobility. The molecular weight of the "38S" would be about 2.1×10^6 daltons.

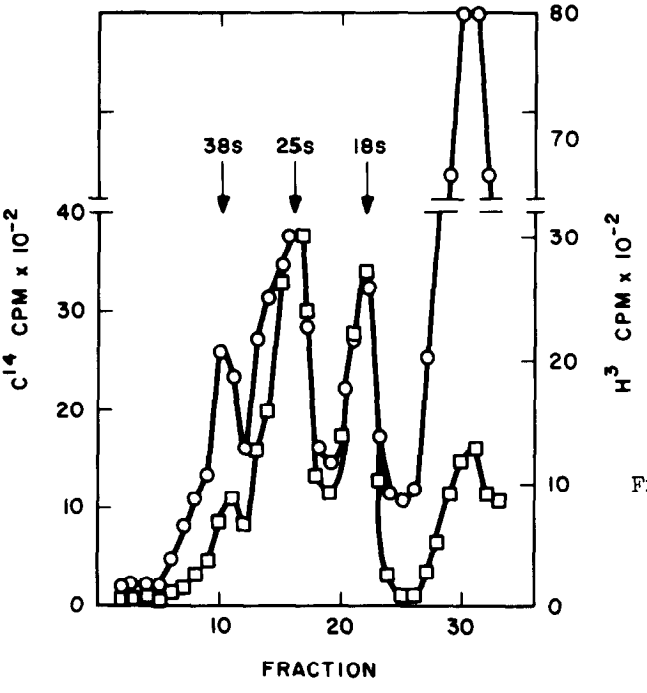

Fig. 3. Comparison of RNA labelled in 15 min. by uracil in Cx treated cells O——O, and control cells □——□. RNA was extracted following mixing of cells from both cultures, and separated on a single sucrose gradient.

That this 38S RNA is a component of normal as well as Cx treated cells is shown in Fig. 3. In this experiment a culture was divided into two aliquots. Cx at the usual concentration was added to one aliquot followed one minute later by H^3 uracil. At the same time C^{14} uracil was added to the control aliquot, and fifteen minutes later both aliquots were collected, pooled and frozen. From this point the preparation was treated exactly as the experiment shown in Fig. 2.

The H^3 (Cx treated) counts show a pattern similar to Fig. 2 with high count regions at both 38 and 4S. It should be noted that the ratio of 38S to 25S counts in this experiment is somewhat reduced from that shown in Fig. 2. The variation shown in these two figures represents the experimental extremes found in six such experiments. From these results we conclude that after fifteen minutes of label there is a small but reproducible 38S count peak in untreated cells. In addition, Cx treatment results in an increased proportion of the $\geq$ 18S counts in the 38 region (Table 1).

The question now arises as to whether this 38S molecule is related to ribosomal RNA. As is shown in Fig. 1, the incorporation of H^3 methyl-methionine parallels the incorporation of uracil after Cx treatment suggesting that metionine methyl groups are incorporated into RNA. This is demonstrated

TABLE I

Distribution of label in RNA synthesized in presence and absence of cycloheximide.

RNA Fraction	% of total RNA		% of RNA 18S or larger	
	Control	Cx	Control	Cx
38S	10	10	13	26
25S	38	16	49	42
18S	29	12	38	32
4S	23	61	--	--

The values given above are calculated from the data in Fig. 3.

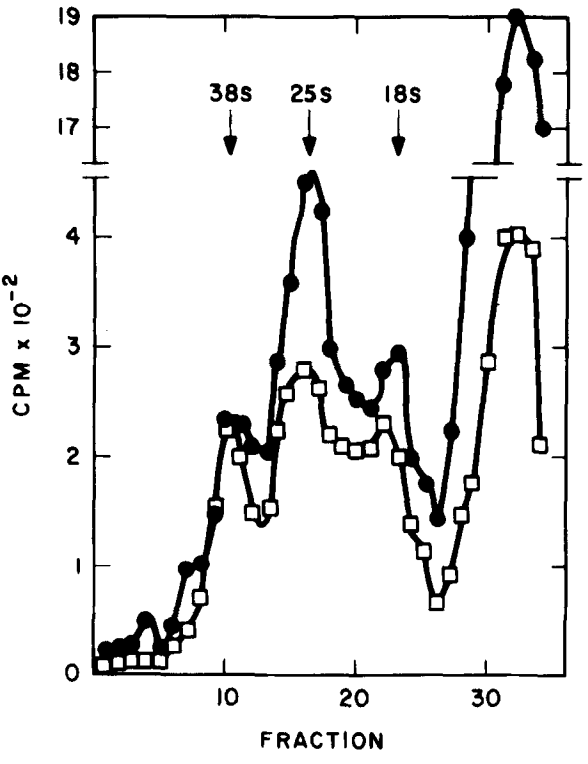

Fig. 4. H^3 Methylation of RNA by H^3 methyl methionine during 15 min. Cx treatment. ●—● H^3 methyl counts in RNA, □—□ C^{14} Uracil. A_{260} values are omitted in this and the previous figure for simplification of figures.

in the experiment shown in Fig. 4. Cx was added to a culture followed one minute later by H^3 methyl-methionine and C^{14} uracil. The cells were collected at fifteen minutes and treated as described above. Methyl H^3 counts as well as uracil counts are found in the 38S region. If the H^3/C^{14} ratio of the 25S peak is normalized to one, the same ratio at 38S is 0.71 and 2.9 at 4S. The fact that the ratio in the 38S region is lower than that of the 25S is similar to the observations of Weinberg et al (1967) who found that in HeLa cells the 45S precursor molecule is less methylated than the mature 18 or 28S molecules. As transfer RNA has been shown to be more methylated than any of the ribosomal species (Borek and Srinivasan, 1966), the high H^3 methyl specific activity in the 4S region indicates that this material is predominantly tRNA and not breakdown products of larger molecules. Moore (1966) has concluded that messenger RNA produced in bacteria is not methylated, and Wagner et al (1967) have shown that only

ribosomal and 4S RNA are methylated in HeLa cells, therefore it appears unlikely that the methyl counts found in the above experiment are due to messenger RNA synthesis.

Our results demonstrate synthesis of rRNA and tRNA during 15 minutes of exposure to Cx in contrast to Fukuhara (1965) who reported only messenger type RNA synthesis in budding yeast following 1 hour Cx treatment. This may be due to prolonged inhibition of protein synthesis affecting control of synthesis and processing of rRNA not yet apparent in the shorter treatment used here.

On the basis of the accumulation during Cx treatment, methylation by methyl labeled methionine, and experiments by others (Loening, Retel and Planta), we conclude that the 38S component is a ribosomal precursor molecule as found in higher eukaryotes. Its accumulation following Cx treatment suggests that a post-transcriptional control mechanism exists in ribosome synthesis.

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