Inhibition of ribonuclease activity during RNA synthesis in isolated yeast nuclei by cadmium

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We have developed an efficient transcription system in isolated yeast nuclei. If $MnCl_2$ is substituted by $CdCl_2$, degradation of newly synthesized RNA is markedly reduced. This effect is due to the inhibition of nuclear ribonuclease activity, since microsomal ribonuclease activity is less affected by the cation. The extent to which the addition of $CdCl_2$ to the in vitro transcription assay inhibits ribonuclease activity is demonstrated by the measurements of the size of newly synthesized RNA. Efficient RNA synthesis in this system is not affected up to a concentration of 0.1 M $CdCl_2$.

Yeast *Cadmium* *RNA synthesis* *Ribonuclease*

1. INTRODUCTION

Cadmium is a potent inhibitor of many enzymes, e.g., $(Na^+\text{-}K^+)$- and $(Ca^{2+}\text{-}Mg^{2+})$-ATPases, and of protein synthesis, and it is a strong uncoupler of oxidative phosphorylation [1,2]. Transcription in isolated rat liver nuclei is inhibited at concentrations of the cation over the range 0.05–100 μM [3], and in vivo RNA synthesis in yeast is completely inhibited at 50 μM $CdCl_2$ [4]. However, cadmium seems to have a different effect on RNA synthesis in isolated yeast nuclei. We report here that $CdCl_2$ stimulates in vitro transcription at concentrations up to 1 mM. Furthermore, degradation of newly synthesized RNA, which usually accompanies transcription in isolated nuclei [5,6], is markedly reduced in the presence of cadmium.

2. MATERIALS AND METHODS

The diploid strain R XII (a kind gift of Dr A. Kotyk, Prague) and the tetraploid strain 2200 [7] of *Saccharomyces cerevisiae* were used. Cells were grown in a medium containing 0.5% peptone, 1% Difco yeast extract and 2% glucose at 28°C and harvested in the fermentative growth phase. For the isolation of nuclei, a modification of the low-temperature procedure in [8] was used. The cells were washed twice with water and then suspended in a buffer consisting of 30 mM Tris–HCl (pH 7.9), 20 mM NaCl, 2 mM $MgCl_2$, 1 mM spermidine, 1 mM spermine, 6 mM 2-mercaptoethanol, 400 mM sucrose and 40% (v/v) glycerol. The cells were kept on ice for 20 min and then broken in a French pressure cell [9]. Following the disruption of the cells the homogenate of maximally 15 g wet wt of cells/40 ml was centrifuged in a Heraeus Christ Minifuge II at 5000 rpm for 15 min at −15°C. The pellet was resuspended in 60 ml buffer and centrifuged under the same conditions. Both supernatants were pooled and centrifuged again as above. Then the supernatant was centrifuged for 15 min at 12000 rpm in an HB-4 rotor (Dupont-Sorvall) at −15°C. The pellet was resuspended in 20 ml buffer and centrifuged for 15 min at 4000 rpm in an HB-4 rotor at low temperature. Finally, the nuclei were pelleted again as above (12000 rpm), resuspended in buffer and stored at −80°C. The DNA content of the nuclei was determined as in [10].

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RNA synthesis in vitro was carried out in a total volume of 250 μl of a reaction mixture containing 50 mM Tris–HCl (pH 7.9), 80 mM K acetate, 5 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM spermidine, 0.2 mM spermine, 6 mM 2-mercaptoethanol, 0.2 mM of each ATP, CTP, GTP and 20 μCi/ml [^{3}H]UTP, and nuclei at a DNA concentration of 50–100 μg/ml. Where indicated, $CdCl_2$ and $MnCl_2$ were added at varying concentrations. The reaction was stopped at different times by adding 5% trichloroacetic acid, the precipitates retained on glass fibre filters, and the filters washed with 5% trichloroacetic acid and ethanol. The radioactivity in the filters was measured in a toluene-based scintillant in a Beckman liquid scintillation counter.

RNA was extracted from the nuclei by a modification of the LiCl/urea procedure [11]. RNA synthesis in vitro (assay volume 500 μl) was stopped by the addition of 3 vols of 4 M LiCl, 8 M urea, 50 mM Na acetate (pH 5.5), 400 μg/ml heparin and 0.1% SDS. The mixture was vortex-mixed until the DNA was dispersed and allowed to stand for 16 h at 4°C. The suspension was centrifuged for 30 min at 10000 rpm at 4°C in an HB-4 rotor, the supernatant discarded and the pellet washed twice with 3 M LiCl, 6 M urea, 50 mM Na acetate (pH 5.5) and 0.1% SDS. The pellet was suspended in 3 ml of 0.2 M Na acetate (pH 5.5) and extracted 3 times with an equal volume of chloroform–isoamyl alcohol (24:1). Then the RNA was precipitated overnight at −20°C with 2.5 vols ethanol. The RNA precipitate was vacuum dried, dissolved in 50 μl sterile distilled water, again precipitated with 2.5 vols ethanol, washed with 0.7 ml of 70% ethanol, dried, and prepared for gel electrophoresis as in [12]. The gels were processed for fluorography as in [13].

A $(NH_4)_2SO_4$ fraction of yeast ribonuclease was prepared from ribosomes as in [9].

3. RESULTS AND DISCUSSION

In the course of our studies on the effect of cadmium on RNA synthesis in yeast, we have developed a cell-free transcriptional system by using isolated nuclei. Because Mg^{2+} and Mn^{2+} were found to be essential for other in vitro transcriptional systems [5,6] these cations were also included in our assay. When $CdCl_2$ was added to this assay, the period during which [^{3}H]UMP was incorporated into RNA was extended. We therefore studied the effect of cadmium on cell-free transcription in yeast. Fig.1 shows the time course of RNA synthesis when nuclei were incubated with $MnCl_2$ or $CdCl_2$ at concentrations of 0.5 and

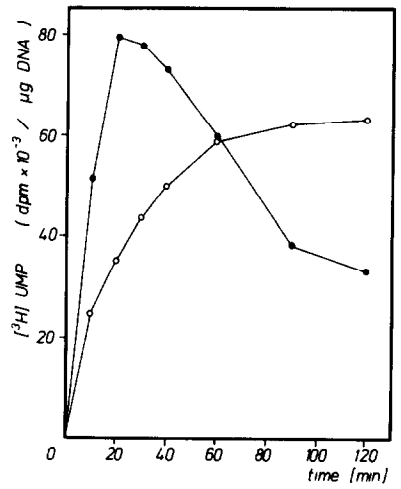

Fig.1. Effect of manganese and cadmium on RNA synthesis in isolated yeast nuclei. Yeast nuclei were incubated for the times indicated at 30°C with 0.5 mM $MnCl_2$ (●—●) or 0.1 mM $CdCl_2$ (○—○).

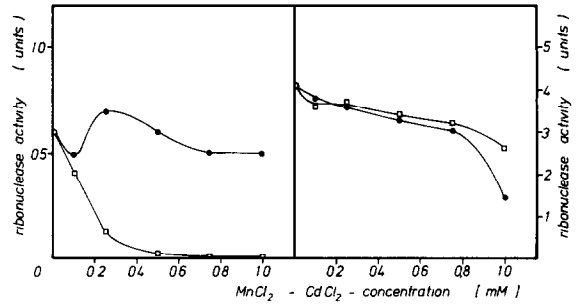

Fig.2. Inhibition of nuclear (A, left) and cytoplasmic (B, right) ribonuclease activities by manganese (●—●) and cadmium (□—□). The cytoplasmic ribonuclease was prepared from yeast ribosomes [9], while nuclear ribonuclease activity was measured in whole yeast nuclei. The ribonuclease activities were assayed in 0.2 ml of a mixture containing 30 mM Tris–HCl (pH 7.5), 50 mM KCl, 1 mM $MgCl_2$, 6 mM 2-mercaptoethanol and 0.5 mg/ml protein of the cytoplasmic fraction or nuclei at a DNA concentration of 150 μg/ml at 35°C. Ribonuclease activity (U): 1 unit = 0.1 $A_{260nm} \cdot min^{-1} \cdot ml^{-1}$.

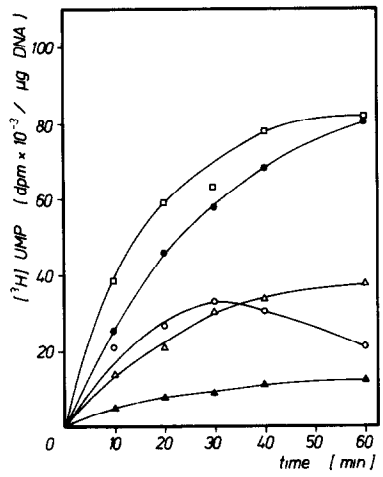

Fig.3. Effect of various cadmium concentrations on transcription in isolated yeast nuclei incubated at 20°C. Control without $CdCl_2$ (○—○), 0.1 mM $CdCl_2$ (△—△), 0.5 mM $CdCl_2$ (●—●), 1 mM $CdCl_2$ (□—□) and 3 mM $CdCl_2$ (▲—▲).

0.1 mM, respectively. In the presence of $MnCl_2$ the nuclei incorporated [^{3}H]UMP during the first 10 min at a very high rate, but after 20 min of incubation, the accumulation of the newly synthesized RNA decreased. In contrast, if $MnCl_2$ was substituted by $CdCl_2$, accumulation of ^{3}H-labelled RNA continued for 60 min. To elucidate whether the decrease of acid-insoluble material is due to

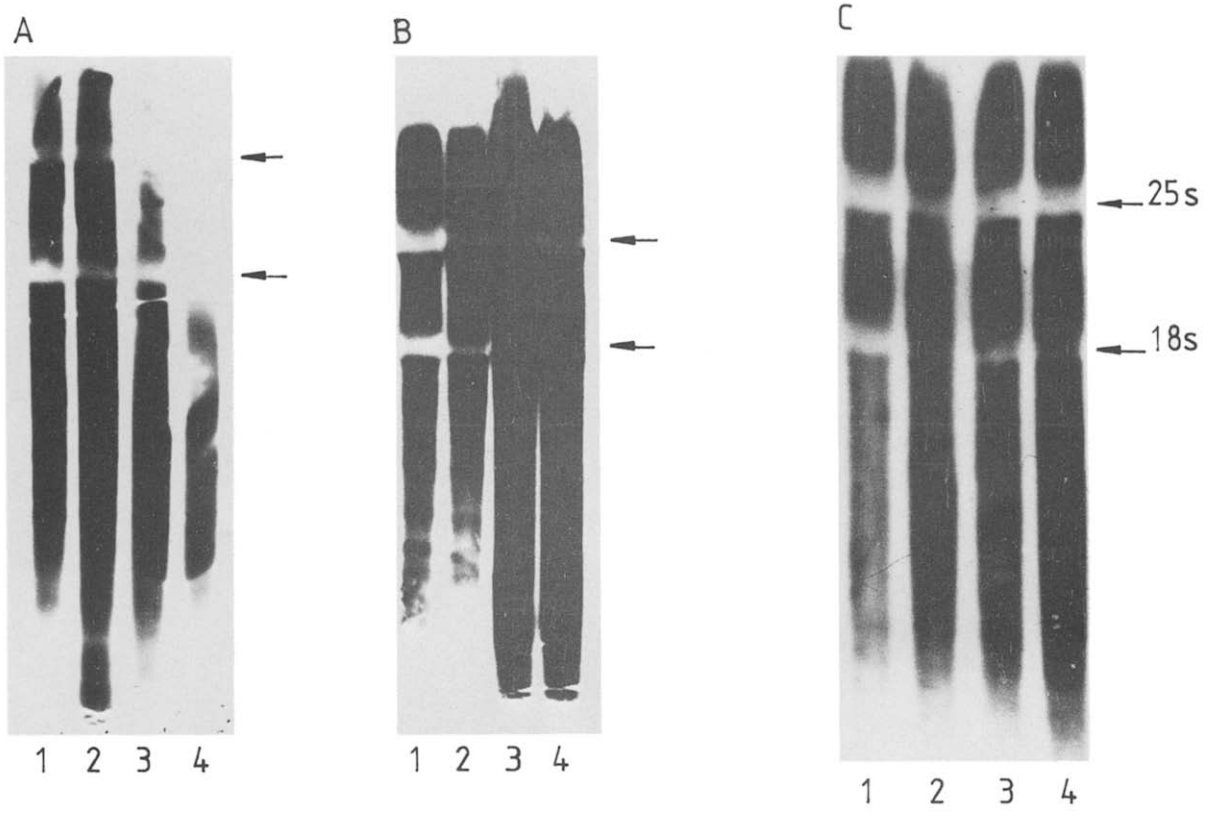

Fig.4. Effect of cadmium on the size of labelled RNA synthesized in vitro by yeast nuclei. The nuclei were incubated, and the RNA was extracted as described in section 2. The size of the labelled RNA was determined by electrophoresis of glyoxal-denatured RNA in 1.5% agarose gels, followed by fluorography. (A) Fluorograph showing in vitro transcripts isolated from yeast nuclei. The nuclei were incubated in the presence of 0.1 mM $CdCl_2$ for 45 min at 20°C (1), 25°C (2), 30°C (3), 37°C (4). (B) Nuclei were incubated in the presence of 1 mM $CdCl_2$ for 45 min at 20°C (1), 25°C (2), 30°C (3), 37°C (4). (C) The nuclei were incubated in the presence of 0.1 mM $CdCl_2$ at 20°C for 20 min (1), 40 min (2), 60 min (3), 90 min (4).

ribonuclease activity, we investigated the effect of the cations on ribonucleases of the nuclear and microsomal fractions. Fig.2A demonstrates that the nuclear ribonuclease was completely inhibited at 0.5 mM $CdCl_2$, whereas the effect of $MnCl_2$ at this concentration was negligible. Although the microsomal ribonuclease was also inhibited by $CdCl_2$ (fig.2B), it is evident that the effect on the nuclear enzyme was considerably greater. Thus, we assume that the reduction of RNA breakdown during transcription is mainly due to an inhibition of nuclear ribonuclease activity. However, the use of cadmium in cell-free transcriptional systems is limited. In accordance with [3] we have also found that transcription in isolated rat liver nuclei is inhibited by cadmium (not shown). Therefore, it is surprising that cadmium enhances the accumulation of in vitro synthesized RNA in yeast nuclei at concentrations up to 1 mM (fig.3).

To demonstrate the extent to which the addition of cadmium to in vitro transcribing nuclei inhibits ribonuclease activity, the size of newly synthesized RNA was determined by agarose gel electrophoresis. Fig.4A,B shows the effect of the incubation temperature on RNA cleavage by ribonucleases in the presence of 0.1 and 1 mM $CdCl_2$. While ribonuclease activity was still detectable at 30 and 37°C in the presence of 0.1 mM $CdCl_2$, with 1 mM $CdCl_2$ the RNA breakdown was also stopped at these high temperatures. However, at 20°C, 0.1 mM $CdCl_2$ was sufficient to prevent RNA degradation over a period of 90 min (fig.4C). Under these conditions, the size of the transcription products is shown to be greater than 25 S. For further characterization we determined the rate of de novo synthesis of mRNA. When the in vitro synthesized RNA was applied to an oligo[d(T)] column, only poly(A^+) RNA could be eluted. This result together with the finding that 10 μg/ml α-amanitin inhibited RNA synthesis only by 5% led us to infer that in our system transcription of mRNA occurred at a very low level (not shown).

Since the synthesis of RNA in cell-free systems is predominantly due to RNA chain elongation by pre-engaged RNA polymerases [5], we decided to measure the activity of free polymerases. In contrast to the ribonuclease inhibitors heparin, aurintricarboxylic acid and vanadyl ribonucleoside complexes, which were found to inhibit free RNA polymerase [6], cadmium permits RNA chain elongation by pre-engaged RNA polymerases and transcription of poly[d(A–T)] by free polymerases in the presence of actinomycin D (not shown).

The use of cadmium in the cell-free transcriptional systems may be restricted to yeast, since in vitro RNA synthesis in a mammalian system was defective [3]. However, it would be of interest to determine whether cadmium is able to enhance the accumulation of newly synthesized RNA in isolated nuclei of plants.

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