THE USE OF D-GALACTOSAMINE FOR A PULSE-CHASE STUDY OF RIBOSOMAL RNA MATURATION IN RAT LIVER

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1. Introduction

The control mechanisms of ribosome biogenesis in animal cells are not yet elucidated ([1] review). Studies with actinomycin D, camptothecin and other inhibitors interacting with DNA or chromatin showed an inhibition of pre-rRNA processing and led to the suggestion that continuous transcription of rRNA genes is directly coupled with the formation and nucleo-cytoplasmic transfer of ribosomes [1,2]. However, the action of actinomycin D on pre-rRNA processing may be independent of its effect on transcription [3] and similar side effects are possible with other drugs interacting with DNA. Therefore, studies with different inhibitors of RNA synthesis, which would not alter chromatin structure, are necessary in order to elucidate the mechanisms controlling pre-rRNA processing and ribosome formation. Experiments with D-galactosamine revealed that this drug has a pronounced 'trapping effect' on free uridine nucleotides in liver [4,5] resulting in a strong inhibition of RNA synthesis [5,6]. These results suggest the use of D-GA for pulse-chase studies on ribosome formation in liver.

In this work we present evidence that D-GA causes within 30 min a complete depletion of free uridine nucleotides in rat liver and a block of RNA synthesis. Under these conditions prelabelled pre-rRNA is processed to mature rRNA species, which migrate from the nucleus to the cytoplasm. These results show the usefulness of D-GA for pulse-chase experiments on RNA processing and suggest that ribosome biogenesis is not directly dependent on continuous transcription of rRNA genes.

Abbreviations: pre-rRNA, precursor(s) to rRNA; HnRNA, heterogeneous nuclear RNA; D-GA, D-galactosamine

2. Experimental

The experiments are carried out with Wistar female albino rats weighing 100 ± 10 g. D-GA at a dose of 250 mg/kg body weight [6] is administered intraperitoneally and labelling in vivo is carried out with [^{14}C]orotate (spec. act. 18 mCi/mmol).

The animals are killed by cervical dislocation, the livers dissected out, rinsed with cold 0.14 M NaCl and processed further in the cold. Detergent-purified nuclei are isolated as described [7]. Three fractions of nuclear RNA corresponding to 'nucleosol', 'nucleolar' and HnRNA are obtained by stepwise extraction with phenol at 4°C, 50°C and 85°C [8,9]. The cytoplasmic fraction is obtained from a liver homogenate in 0.32 M sucrose, 0.01 M Tris–HCl (pH 7.0), 0.005 M $MgCl_2$ and 1% Triton X-100 after sedimentation of nuclei for 10 min at 2000 × *g*. The cytoplasmic RNA is extracted with phenol at 4°C [10]. The RNA fractions are freed from low molecular weight components and free nucleotides by precipitation with 1.5 M NaCl, followed by passage through a column of Dowex 1, $HCOO^-$ form.

The nuclear and cytoplasmic RNA fractions are analysed by agar–urea gel electrophoresis according to [11]. The labelling of the separate RNA fractions is determined by cutting the dried agar gel film in 1 mm slices and solubilisation of the RNA components with 0.5 ml 2.5% NH_4OH per sample for 24 h at room

temperature. To each sample are added 4.5 ml mixture containing 1 vol. Triton X-100 and 2 vol. toluene/PPO/DimethylPOPOP phosphor [12] and the radioactivity determined in a Packard TriCarb 3320 scintillation spectrometer.

The amount of uridine nucleotides and their derivatives in liver is determined by two-dimensional paper chromatography as described [13,14].

3. Results and discussion

Analysis of free uridine nucleotides and their derivatives (table 1) reveals a four-fold decrease of UTP at 15 min after D-GA, while at 30 min all uridine nucleotides, as well as UDP-glucose and UDP-glucuronic acid are totally depleted. The fast 'trapping effect' of D-GA is demonstrated by the rapid accumulation of UDP-*N*-acetylglucoseamine and UDP-hexoseamines [5,15].

The inhibition of HnRNA and rRNA synthesis by D-GA is studied by 10 min in vivo labelling with [^{14}C]orotate given at different time intervals after D-GA (fig.1). The results show that the synthesis of both HnRNA and rRNA is reduced to background levels as early as about 20 min after D-GA-administration.

The observed rapid block of RNA synthesis caused

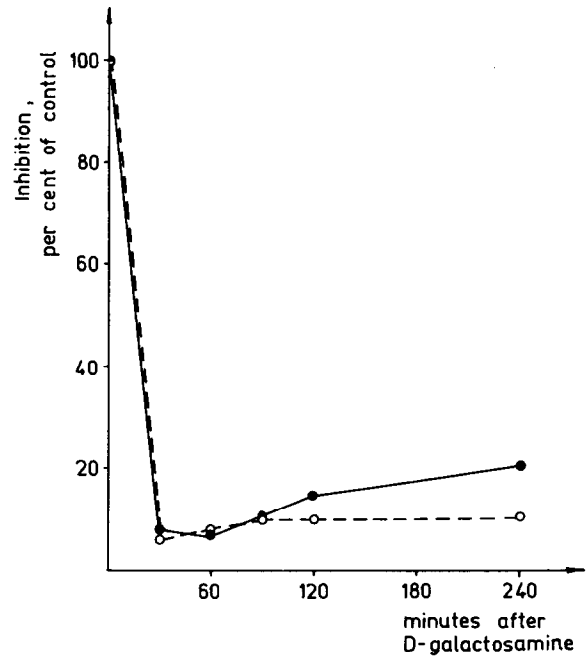

Fig.1. Inhibition of [^{14}C]orotate incorporation into rat liver nucleolar 50°C RNA (o——o) and heterogeneous nuclear 85°C RNA (o- - -o) after D-GA. Female albino rats (100 g body wt, 3 per point) are given 25 μCi [^{14}C]orotate per animal and killed after administration of 25 mg/animal of D-GA. The nuclear RNA fractions are isolated and their specific radioactivity determined as described in Experimental.

Table 1
Amount of free uridine nucleotides and UDP-sugars in rat liver after D-galactosamine[a]

Compound	Time after D-galactosamine administration			
	Controls	15 min	30 min	60 min
UTP	16.6 ± 0.9	4.3 ± 0.1	0	0
UDP	7.9 ± 1.6	5.1 ± 0.1	0	0
UMP	16.3 ± 0.9	6.7 ± 0.2	0	0
UDP-hexoses	18.3 ± 3.0	3.2 ± 0.2	0	0
UDP-*N*-acetyl-hexosamine	26.2 ± 2.0	28.3 ± 1.2	29.8 ± 2.0	36.5 ± 7.0
UDP-hexuronic acids	24.3 ± 3.0	7.3 ± 0.2	5.2 ± 0.1	0
UDP-hexosamines	0	33.0 ± 4.0	56.0 ± 6.0	71.7 ± 6.3

[a] The amount is given as μmol/100 g wet wt liver ± standard error

The rats are treated with 250 mg/kg D-galactosamine given intraperitoneally. The amount of uridine derivatives in liver is determined as described in Experimental. Each point is the average from 8 experiments

by D-GA make possible the use of this drug for a pulse-chase study of pre-rRNA maturation at time periods when its general toxic effects are not yet displayed [6]. Therefore, we carried out experiments in which RNA is labelled with [^{14}C]orotate for 10 min in vivo, followed by D-GA action for 60 min, 90 min and 120 min. The phenol fractionation technique for nuclear RNA used here has the advantage to yield a fraction containing all nucleolar pre-rRNA and rRNA components free of HnRNA contamination [9], although the structure of nucleoli is destroyed [6] and their isolation is made impossible. The 'nucleolar' RNA fraction is analysed by agar–urea gel electrophoresis (fig.2). The results show that in control rats all nucleolar pre-rRNA and rRNA components form neatly delineated ultraviolet peaks, the bulk of the label at 10 min being located in 45 S pre-rRNA [12]. At 60 min after D-GA the label is shifted towards 32 S and 21 S pre-rRNA, as well as into mature nuclear 28 S and 18 S rRNA. At longer periods of D-GA action the label is mainly in 28 S rRNA. It is noteworthy that D-GA causes the disappearance of the ultraviolet peaks of all nucleolar pre-rRNA components. The ultraviolet peak of nuclear 28 S rRNA is also decreased, but quantitative estimates of these changes are not possible due to contamination of nuclear rRNA components by unlabelled rRNA of cytoplasmic origin [10].

The shift of prelabelled pre-rRNA and the disappearance of pre-rRNA ultraviolet peaks demonstrate that the block of RNA synthesis by D-GA does not alter intranuclear processing or pre-rRNA. The possi-

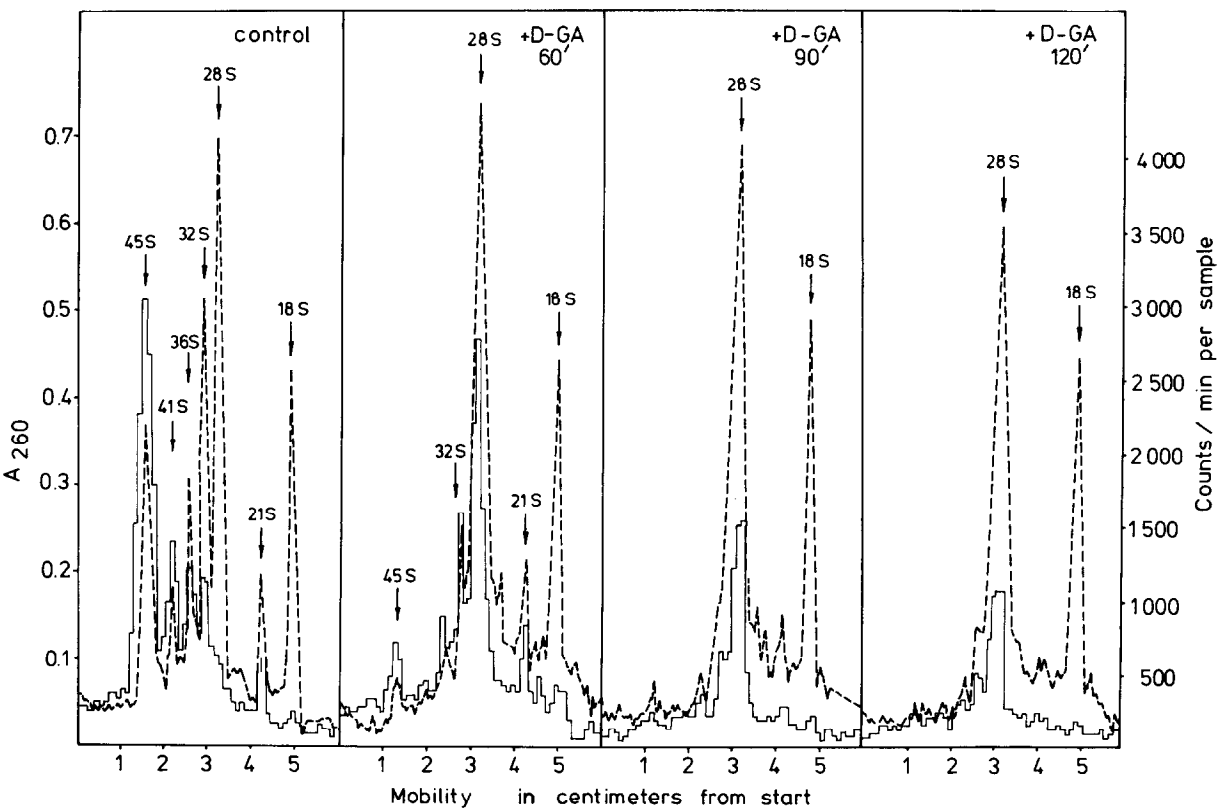

Fig.2. Agar–urea gel electrophoresis of rat liver nucleolar 50°C RNA after 20 min labelling with [^{14}C]orotate (control) and 60 min, 90 min and 120 min chase with D-GA. The experimental animals (5 female rats/group of 90 g body wt each) are given 15 μCi [^{14}C]orotate and 20 min later 250 mg/kg D-GA. The nucleolar 50°C RNA fraction is isolated and analysed as described in Experimental. (—) Radioactivity; (- - -) $A_{260\,nm}$.

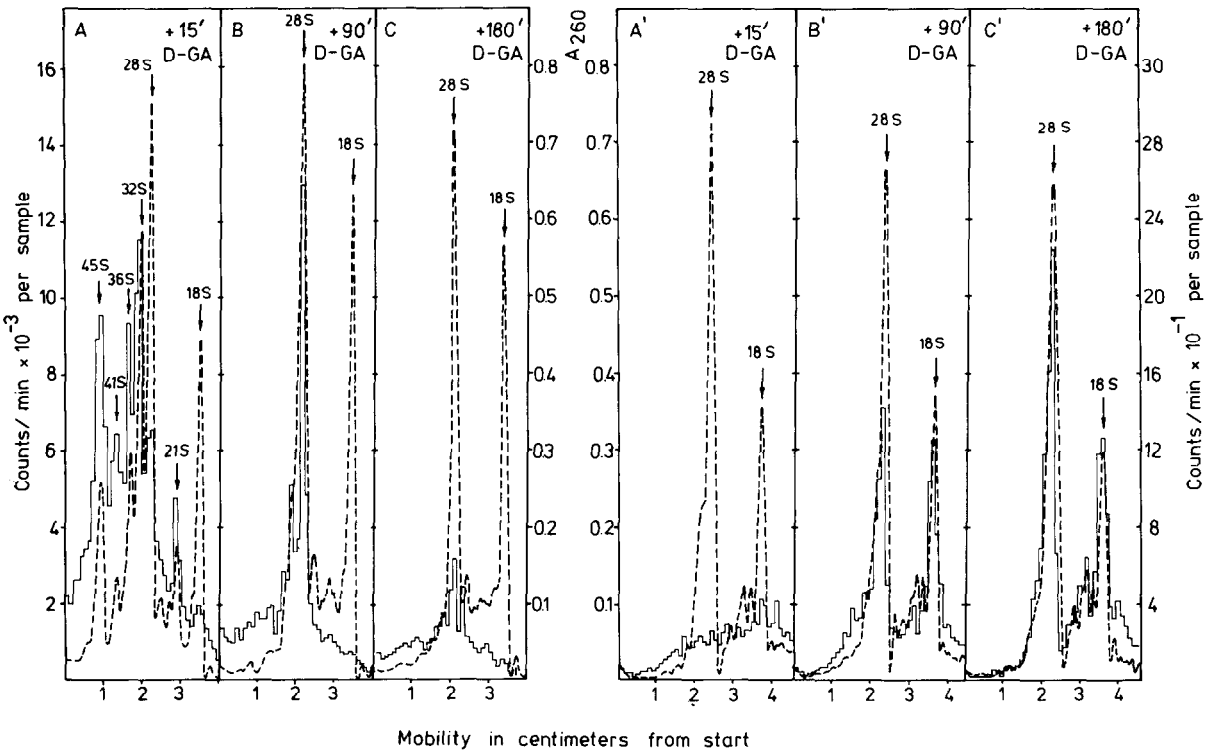

Fig.3. Agar--urea gel electrophoresis of rat liver nucleolar 50°C RNA and cytoplasmic RNA after 30 min labelling with [^{14}C]-orotate and 15 min, 90 min and 180 min chase with D-GA. The experimental animals (5 female rats/group of 120 g body wt each) are given 50 μCi [^{14}C]orotate and 30 min later 250 mg/kg D-GA. The nucleolar 50°C RNA and cytoplasmic RNA fractions are isolated and analysed as described in Experimental. (A), (B) and (C) – nucleolar 50°C RNA after 15, 90 and 180 min chase; (A′), (B′) and (C′) – cytoplasmic RNA after 15 min, 90 min and 180 min chase. (----) Radioactivity; (- - -) A_{260} nm.

bility that under conditions of D-GA block pre-rRNA species are unspecifically degraded is unlikely since the peaks of nuclear rRNA do not show even incipient degradation [16] and labelled low molecular weight RNA components are not detected on the electrophoregrams (data not shown). Thus the gradual decrease of nucleolar 28 S rRNA labelling upon D-GA treatment suggests that under these conditions unhampered nucleo-cytoplasmic transfer of pre-labelled nuclear rRNA is taking place.

In order to verify this assumption experiments were carried out where nuclear pre-rRNA is pre-labelled in vivo for 30 min before D-GA administration. The analyses of the nucleolar and cytoplasmic RNA fractions [8–10] presented in fig.3 show that after D-GA block of RNA synthesis:

(a) The bulk of pre-labelled pre-rRNA is processed to mature 28 S and 18 S rRNA.
(b) The mature 28 S and 18 S rRNA molecules are transferred from the nucleus to the cytoplasm.
(c) The appearance of labelled 18 S rRNA in the cytoplasm precedes that of 28 S rRNA.

In particular, a block in the nucleo-cytoplasmic transfer of 28 S rRNA as caused by camptothecin [2] is not observed under D-GA inhibition of total RNA synthesis.

The obtained results suggest that processing of pre-rRNA and ribosome biogenesis are not directly dependent on continuous transcription of nucleolar and nucleoplasmic genes. Therefore, previous conclusions about the direct coupling of transcription with proces-

sing and nucleocytoplasmic transfer of rRNA, derived from studies with actinomycin D,camptothecin and similar inhibitors interacting with DNA or chromatin (see [1]) cannot be considered of general significance and may reflect differences in the response of various cell types or some side effects of these drugs. The role of the supply of structural and/or regulatory proteins in the control of ribosome biogenesis [1] is not ruled out by our present results. However, they indicate that at short terms of D-GA action, the synthesis and the supply of these putative control proteins is not altered [6,17] to an extent capable to cause a block in ribosome biogenesis.

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