EFFECTS OF CORTISOL ON RNA SYNTHESIS AS DETECTED BY HYBRIDIZATION WITH DIFFERENTIALLY RENATURING DNA SPECIES*

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1. Introduction

In recent years evidence has been presented that administration of cortisol results in an increased synthesis of RNA in rat liver nuclei [1, 2]. On the basis of hybridization-competition experiments, Drews and Brawerman [3] proved that cortisol causes the appearance of new species of RNA in rat liver nuclei. Recent studies of Britten and Kohne [4] on the re-association kinetics of DNA gave evidence that DNA of higher organisms is composed of different types of sequences with respect to their renaturation kinetics. From these experiments, they postulated the existence of "highly repetitive" and "unique" sequences. In this communication we present experimental data describing the hybridization capacity of nuclear RNA from both control and cortisol-treated rats with differently renaturing species of DNA

2. Materials and methods

Male Wistar BR II rats, weighing 120 g were used. Cortisol was kindly provided by Schering AG, Berlin. Triton X 100 was purchased from Serva, Heidelberg, ^{3}H-orotic acid (sp. act. 22.3 Ci/mmole) from the Radiochemical Centre, Amersham and ribonuclease (DNAase-free, E.C. 2.7.7.17) from Worthington Biochem. Corp. All other chemicals were from Merck AG, Darmstadt. Nitrocellulose filters (SM 11306, diameter 27 mm) were obtained from Sartorius Membranfilter, Göttingen. Pronase (B grade) was from Calbiochem.

2.1. *Preparation of DNA*

Chromatin was prepared by the method of Marushige and Bonner [5]. It was then homogenized in a buffer at pH 8 containing 2 M NaCl, 5 M urea, 0.01 M tris, 0.001 M EDTA, 0.001 M mercaptoethanol and 0.001 M $MgCl_2$. The homogenate was centrifuged for 15 hr at 65,000 rpm in a Beckman Al 65 rotor. By this treatment, 95% of the chromosomal proteins are removed. The sediment was dissolved in 0.1 × SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and then treated as described by Marmur [6] including treatment with RNAase (5.0 mg/100 ml 0.14 M NaCl) and subsequently pronase (2.5 mg/100 ml 0.14 M NaCl) which was previously autodigested at 37°C for 2 hr, (RNAase treatment 30 min, pronase 6 hr at 37°).

2.2. *Preparation of RNA from control and hormone treated rats*

Cortisol (2 mg/100 g body weight in 0.5 ml of 0.14 M NaCl) was injected intraperitoneally; control rats received 0.5 ml of 0.14 M NaCl. 50 min later a 10-min pulse of ^{3}H-orotic acid (100 μCi/100 g body weight) was administered to both control and cortisol-treated rats. Rats were killed by cervical dislocation. Rapidly labeled RNA was then prepared as previosuly described [7].

2.3. *Reassociation curve of DNA*

DNA was dissolved in 0.12 M phosphate buffer (PB) and sheared for 30 min in a MSE sonifier (output 2.0; 0.8 mA). By this treatment, DNA was

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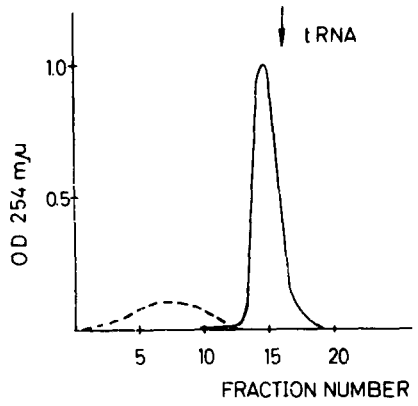

Fig. 1. Density gradient centrifugation of sheared rat liver DNA. Centrifugation was performed by layering 0.5 ml of DNA solution on 5 to 30% w/v linear sucrose gradients at 30,000 rpm for 15 hr in a Beckman SW 41 rotor. (—— sheared DNA, – – – unsheared DNA).

broken to fragments sedimenting at 5 S (see fig. 1).

Hydroxyapatite was prepared by the method of Levin [8]. Denaturation was performed in a boiling water bath for 10 min in 0.12 M PB. After incubation for a given period of time at 60°, the DNA was fractionated on a water-jacketed hydroxyapatite column (8 × 0.8 cm) at 60° as proposed by Bernardi [9]. Double stranded DNA was eluted by 0.4 M PB. DNA concentration in the eluate was estimated by optical density; reassociation was expressed as percentage of renatured DNA versus C_0t (see fig. 2).

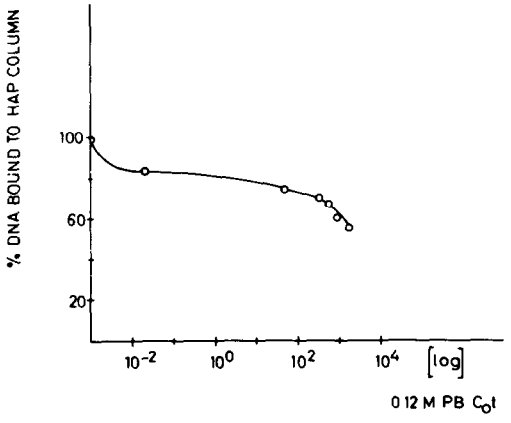

Fig. 2. Reassociation curve of sheared rat liver DNA.

2.4. *Hybridization*

For hybridization, DNA from the following parts of the reassociation curve was used: DNA renaturing immediately (10 sec) after denaturation (fraction I), DNA renaturing 4 hr after denaturation (II), DNA remaining single stranded even after 75 (III) and 100 (IV) hr of incubation at 60°. (C_0t 3×10^{-2}, 4×10, 5.4×10^2, 8.1×10^2; C_0t = moles of nucleotides/l/sec).

Prior to the preparation of filters, DNA was again thermally denatured and chilled by decanting the boiling sample into a tube containing 1.5 volumes of frozen 10 × SSC, the DNA solution thus being cooled down immediately to 3–5°. All the denatured fractions, including the highly repetitive ones, showed a relative increase in O.D. of about 22%. Similar results could be obtained by alkaline denaturation.

With this single stranded DNA, filters were prepared according to Gillespie and Spiegelman [10], each filter bearing 4 μg of DNA. Hybridization was performed by incubating the filters, bearing different kinds of DNA, with 100 μg (near saturation level) of nuclear RNA from control or cortisol-treated rats for 40 hr in 5 ml 6 × SSC at 66°. Prolongation of the incubation time to 65 hr did not result in a rise in hybridization values. After incubation, the filters were washed by suction-filtration with 6 × SSC, treated for 60 min at room temperature with RNAase (20 μg/filter) and washed again by suction filtration with 6 × SSC from both sides. After drying, the filters were counted in a scintillation fluid containing 200 mg POPO and 5 g PPO in 1 l toluene with a Nuclear Chicago Mark I liquid scintillation counter.

3. Results and discussion

Sonication of DNA for 30 min shears DNA to fragments sedimenting in the range of 5 S. Fractionation of this sheared DNA from rat liver on hydroxyapatite by the method of Bernardi [9] gives a reassociation curve as presented in fig. 2. At C_0t values of 10^3, renaturation became extremely slow indicating that a considerable fraction of the DNA investigated

in our system consists of unique sequences (around 50%). These results are consistent with those of Davidson and Hough [11].

Hybridization experiments were then performed employing DNA showing different reassociation kinetics (fractions I–IV, see fig. 2) and RNA from control and cortisol-treated rats. As seen from fig. 3, nuclear RNA from cortisol-treated rat livers differs from control RNA in its hybridizing behaviour with various kinds of DNA. Control RNA hybridizes preferentially with the rapidly reassociating fraction of the genome whereas the nuclear RNA from cortisol-treated rats hybridizes preferentially with DNA renaturing after incubation at 60° in 0.12 M PB for 4 hr.

The usual procedure of performing DNA-RNA hybridization experiments is to anneal unfractionated DNA with the RNA to be examined. These experiments cannot differentiate between DNA species with respect to reassociation kinetics. Britten and Davidson [4] using the hydroxyapatite method of Bernardi [9] were able to resolve sheared DNA into different species, some of which renature very rapidly (repetitive sequences) whereas others show a very slow renaturation kinetic (unique fraction). It was therefore of interest to examine the hybridization capacity of newly formed RNA induced by different simuli with these different DNA species.

It is well established that cortisol treatment leads to increased RNA synthesis in the liver [1]. Part of this newly synthesized RNA is DNA-like (unpublished observations and [12]) and is degraded up to 95% within the nucleus and has therefore been implicated as taking part in intranuclear regulatory processes. Recently, Britten and Davidson [13] and Georgiev [14] have proposed models for genetic control in higher organisms in which sizeable portions of the nuclear RNAs play a central regulatory role. Administration of cortisol, parallel to the induction of other types of RNA, could stimulate such regulatory nucleic acids.

The results presented above seem to be the first indication that cortisol induces the synthesis of RNAs differing with respect to their hybridization capacity towards different kinds of DNA regarding renaturation kinetics. A sharp increase in hybridization can be observed towards a part of the genome which represents an intermediate between highly repetitive and

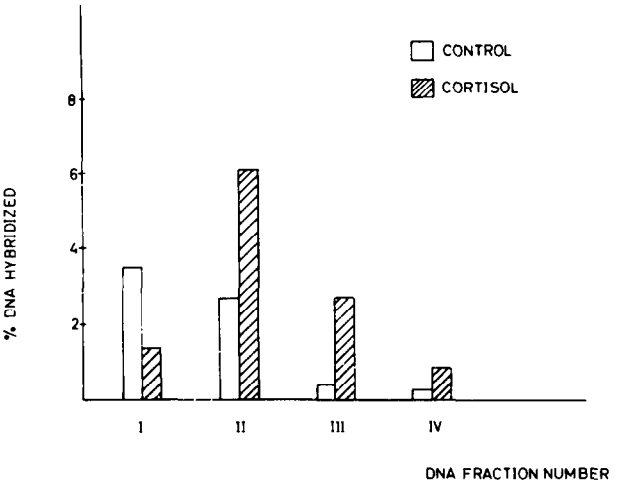

Fig. 3. Hybridization of RNA from control and cortisol-treated rats with different DNA species. The labeled RNA had a specific activity between 100 and 200 cpm/μg RNA. For hybridization conditions see methods. DNA fractions were obtained at C_0t : 3×10^{-2} (fraction I), 4×10 (II) 5.4×10^{-2} (III) and 8.1×10^{2} in 0.12 M PB.

unique sequences. A significant increase in hybridization capacity over control RNA could also be observed at unique sequences of the genome. Similar results have been described by Davidson and Hough [11] demonstrating the synthesis of diverse RNAs during the lampbrush stage of the developing oocyte of Xenopus.

It is still too early to interpret these findings in terms of the proposed models. Nevertheless the experimental data presented above, which lend proof to a specific action of cortisol on genetic transcription, should be further explored and elaborated.

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