A PROPOSED MODEL FOR THE GLUCOCORTICOIDAL REGULATION OF RAT HEPATIC RIBOSOMAL RNA SYNTHESIS

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SUMMARY

Cortisone evokes increased hepatic ribosomal RNA synthesis in vivo. Nuclei and nucleoli isolated from livers of such hormone treated animals manifest enhanced rates of ^{14}C-GTP incorporated into RNA in vitro in the presence of endogenous or exogenous deoxynucleotide templates (PNAS 68, 2177, 1971). When α-amanitin, which inhibits nucleoplasmic gene transcription, was given in vivo concurrently with cortisone, the hormonal elevation of nuclear and nucleolar RNA synthesis was abolished. These findings, in conjunction with earlier evidence that a catalytically essential polypeptide component of nucleolar RNA polymerase I is hormonally inducible, suggests that glucocorticoidally enhanced transcription of the nucleoplasmic genome coding for one or more polypeptide moieties of the nucleolar RNA polymerase I system may be responsible for the hormonal modulation of hepatic ribosomal RNA synthesis.

INTRODUCTION

In eukaryotic cells ribosomal RNA (rRNA), is over eighty percent of the total cellular RNA (1). It originates in the nucleolus as a 45S ribosomal precursor RNA molecule which undergoes hydrolytic nuclear processing and maturation finally generating the 28S and 18S ribosomal RNA species which become ultimately incorporated into the cytoplasmic 60S and 40S ribosomal subunits (1). Multiple nuclear RNA polymerase species have been recently solubilized and identified (2-4). As the nucleolus contains both the genome complimentary to ribosomal RNA (5), and the RNA polymerase I which transcribes this genome, it is presumably the subcellular site responsible for the transcriptional synthesis of ribosomal RNA (6-8). The rate of synthesis of ribosomal RNA is subject to a wide variety of physiological and hormonal influences (9-18). The biochemical mechanism(s) underlying this regulation is, in general, unknown. It is conceivable that various physiological parameters may alter the rates of ribosomal RNA synthesis by either of two distinctive mechanisms: a)controlling availability of the DNA template by regulating the degree of nucleolar gene amplification or the degree of derepression of the nucleolar genome coding for ribosomal RNA or, b)controlling the activity of the ribosomal RNA polymerase I which catalyzes the transcriptive synthesis of ribosomal precursor RNA. We have attempted

to explore the regulation of rat hepatic ribosomal RNA synthesis by cortisone, particularly to distinguish between these alternative regulatory processes.

Evidence has been accumulated from our (9-11) as well as other (19-21) laboratories, over the years to indicate that one of the dramatic metabolic alterations evoked by the in vivo administration of glucocorticoids is stimulation of rat hepatic rRNA synthesis. Recently, we have reported that isolated hepatic nucleoli retain the capacity for accelerated rates of polyribonucleotide synthesis in vitro which reflect their in vivo stimulation (22). Furthermore, we found that the stimulated rRNA synthesis after in vivo cortisone treatment was due largely to increased RNA polymerase I activity per se rather than to increased availability of the ribosomal RNA genome (22). Supporting the physiological import of this observation was the finding (23) that at least one polypeptide component of the nucleolar RNA polymerase I complex, i.e., a protein required for RNA polymerase I activity, was metabolically unstable with an estimated biologic half-life time of only 1.3 hours, indicating that the functional tissue level of this enzyme system would be rapidly responsive to altered translational rates (23). Now we wish to report further studies which in conjunction with the previous findings (22, 23), support the following hypothesis for the glucocorticoid control of hepatic ribosomal RNA synthesis:

a)the stimulation of rat hepatic ribosomal RNA synthesis by cortisone is neither due to the derepression of the ribosomal RNA genome, nor to allosterically increased catalytic efficiency of the pre-existing ribosomal RNA polymerase, but rather is a consequence either of the increased synthesis of one or more catalytically essential protein component(s) of the core ribosomal RNA polymerase I per se., or of one or more polypeptide factor(s) required for its transcriptive function;

b)ongoing transcriptive function of RNA polymerase II, the nucleoplasmic RNA polymerase, is required to mediate this hormonal stimulation of ribosomal RNA polymerase I activity.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing about 150-200 grams, were starved for 48 hours and divided into four groups. Cortisone acetate suspension (Upjohn Co.), 5 mg/100 g body weight, cycloheximide (Acti-Dione, Mann Research Lab), 3 mg/100 g body weight and α-amanitin (Henley and Co., Inc.), 50 µg/100 g body weight were injected intraperitoneally either alone or in combination with the hormone 3 or 4 hours, as indicated in the legend, before they were sacrificed. The livers were perfused with cold 0.25 M sucrose - 3.3 mM $CaCl_2$ solution, homogenized and the nuclei and nucleoli isolated as previously described (22). The nucleolar RNA polymerase activity was assayed in vitro in a low ionic strength Mg^{+2} reaction mixture as previously described (23). The RNA polymerase activities depicted represent triplicate determinations in 2-4 replicate experiments.

TABLE I

EFFECT OF CYCLOHEXIMIDE ON CORTISONE INDUCED RIBOSOMAL RNA POLYMERASE ACTIVITY

FRACTION	CORTISONE*	CYCLOHEXIMIDE**	pMOLE OF [^{14}C] GMP INCORPORATED /mg DNA	%
Nuclear	-	-	692	100
	+	-	1045	151
	-	+	232	34
	+	+	229	33
Nucleolar	-	-	2947	100
	+	-	6704	227
	-	+	429	15
	+	+	483	16

* Cortisone acetate suspension (Upjohn Co.), 5 mg/100 g body weight was given intraperitoneally 3 hours (nuclear fraction) or 4 hours (nucleolar fraction) before the animals were killed.

** Cycloheximide (Acti-Dione, Mann Research Lab., Inc.), 3 mg/100 g body weight, was given intraperitoneally always at the same time as cortisone was given.

TABLE II

EFFECT OF α-AMANITIN ON CORTISONE INDUCED RIBOSOMAL RNA POLYMERASE ACTIVITY

FRACTION	CORTISONE*	α-AMANITIN**	pMOLE OF [^{14}C] GMP INCORPORATED /mg DNA	%
Nuclear	-	-	672	100
	+	-	1308	195
	-	+	723	108
	+	+	681	101
Nucleolar	-	-	2707	100
	+	-	6312	233
	-	+	425	16
	+	+	397	15

* Cortisone acetate, 5 mg/100 g body weight, was injected intraperitoneally 3 hours before the animals were killed

** α-Amanitin (Henley and Co., Inc.), 50 μg/100 g body weight, was also injected intraperitoneally 3 hours before the animals were killed.

RESULTS AND DISCUSSION

Treatment of rats with cortisone for either 3 or 4 hours increases hepatic RNA polymerase I activity over that of the controls (Table I). Furthermore, as reported earlier (22), this elevated RNA polymerase activity is localized largely in the nucleolar fraction (Table I). As shown in Table I,

cycloheximide, 3 mg/100 g body weight given intraperitoneally, an amount known to be completely effective in inhibiting protein synthesis for at least 4 hours after a single injection (23), essentially eliminates the cortisone enhancement of ribosomal RNA synthesis. This result, in conjunction with previous studies (22, 23), supports the belief that the stimulation of ribosomal RNA synthesis by cortisone is due to neither a derepression of the ribosomal RNA genome, nor an increased catalytic efficiency of the pre-existing enzyme (24), but rather results from an increased rate of synthesis of this core enzyme or of a protein factor required by it. These results also exclude the possibility that cortisone might increase the enzyme concentration by decreasing the rate of degradation of the pre-existing enzyme.

Blatti *et al.* (25), and Sajdel *et al.* (24), found that after glucocorticoid treatment, although the nucleoplasmic RNA polymerase II activity was not changed, the nucleolar RNA polymerase I activity was selectively augmented. Since these investigators studied solubilized polymerase preparations, assayed using exogenous template, they also concluded that hormonal regulation of ribosomal RNA synthesis is not mediated by increasing the availability of the DNA template, but rather by regulating the level of catatlytic efficiency of the RNA polymerase I *per se*. Sajdel *et al.* (24), to account for the increased activity, proposed that the hormone induced an allosteric change in the nucleolar RNA polymerase activity. This interpretation is not supported by the present findings indicating that ongoing protein synthesis is required for the increase in ribosomal RNA polymerase activity. Furthermore, as is shown in Table I, after cycloheximide treatment, the RNA polymerase I activity decays rapidly below control values. This is in good agreement with previous findings (23) and indicates that the nucleolar RNA polymerase I or a protein component required for its catalytic activity is one of the most rapidly turning over of the hepatic proteins with an estimated half-life time of 1.3 hours.

These studies suggested that the stimulation of ribosomal RNA synthesis by cortisone is a result of increased rate of synthesis of one or more polypeptide components of the RNA polymerase I system. Two alternative processes might account for these results: a)the hormone may, by an unspecified mechanism, specifically accelerate the translational synthesis of this hormonally inducible protein or, b)the hormone may act transcriptionally at a locus distinct from the ribosomal RNA genome (22), to produce more messenger RNA coding for the synthesis of the polypeptide component(s) of RNA polymerase I system. α-Amanitin was employed to distinguish between these alternatives. This cyclic octapeptide from the poisonous mushroom *Amanita phalloides* (26), is known to specifically inhibit the nucleoplasmic

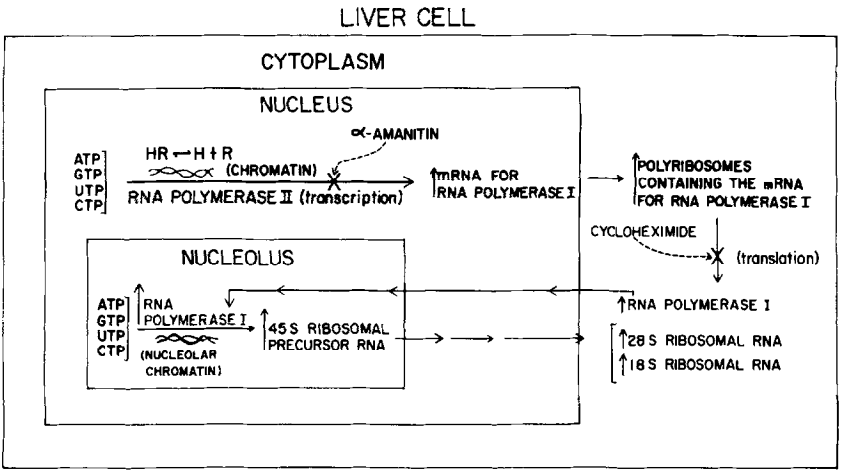

Fig. 1.

A proposed model for the glucocorticoid regulation of rat hepatic ribosomal RNA synthesis.

RNA polymerase II (4, 27, 28). Since RNA polymerase II is presumed to be responsible for transcription of the extranucleolar genome and for messenger RNA synthesis, we reasoned that if cortisone acts translationally then α-amanitin treatment in vivo should not block the hormonal effect; should α-amanitin block glucocorticoidal induction of elevated RNA polymerase I levels, this would suggest that a transcriptive process involving RNA polymerase II is required to manifest the hormonally stimulated ribosomal RNA polymerase I activity. When α-amanitin, 50 μg/100 g body weight, was given in vivo concurrently with the hormone, the stimulatory effect by cortisone on the ribosomal RNA polymerase activity was entirely abolished (Table II). We, therefore, concluded that the hormonal stimulation of ribosomal RNA synthesis requires, not only protein synthesis but also nucleoplasmic RNA polymerase II mediated messenger RNA synthesis.

Figure 1 summarizes the major steps involved in the biosynthesis of ribosomal RNA in eukaryotic cells (1) and depicts one hypothesis concerning the events by which the glucocorticoid may regulate ribosomal RNA synthesis in hepatocytes. The hormone (H), presumably as the hormone-receptor protein complex (HR) (29-33), may derepress or activate a specific locus on the extranucleolar chromatin augmenting the transcriptional synthesis of messenger RNA(s) coding for one or more of the polypeptide components of the RNA polymerase I system. This process requires the participation of the α-amanitin sensitive RNA polymerase II (Table II). The enhanced levels of the messenger RNA(s) for the RNA polymerase I system are then translated in the cytoplasm in a cycloheximide sensitive process (Table I). The hormonally induced

polypeptide(s) which may be core components of RNA polymerase I, or factors required for its catalytic function, enter the nucleolus where the elevated levels of functional RNA polymerase I act on the nucleolar chromatin with enhanced production of 45S ribosomal precursor RNA leading ultimately to augmented levels of the 28S and 18S cytoplasmic ribosomal RNA species. Thus, the glucocorticoid mediated enhancement of ribosomal RNA synthesis seems due, not to hormonal derepression of the nucleolar genome coding for ribosomal RNA but, rather, to hormonal derepression of the nucleoplasmic genome coding for polypeptide(s) which may be either components of the RNA polymerase I core enzyme or are required for its catalytic activity.

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