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Multihormonal induction of hepatic α_{2u}-globulin mRNA as measured by hybridization to complementary DNA

(mRNA purification/preparative hybridization/transcriptional control)

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ABSTRACT A procedure is presented for the preparation of a ^{3}H-labeled complementary DNA (cDNA) specific for the mRNA coding for α_{2u}-globulin, a male rat liver protein under multihormonal control that represents approximately 1% of hepatic protein synthesis. Rat liver polysomes are incubated with monospecific rabbit antiserum to α_{2u}-globulin, which binds to the nascent α_{2u}-globulin chains on the polysomes. These antibody–polysome complexes are then adsorbed to goat antiserum to rabbit IgG that is covalently linked to *p*-aminobenzylcellulose. mRNA preparations are thus obtained that contain 30–40% α_{2u}-globulin mRNA. A labeled cDNA is made to this α_{2u}-globulin-enriched mRNA preparation by using RNA-dependent DNA polymerase (reverse transcriptase). To remove the non-α_{2u}-globulin sequences, this cDNA preparation is hybridized to an RNA concentration × incubation time (R_0t) of 1000 mol of ribonucleotide per liter × sec with female rat liver mRNA, which, though it shares the vast majority of mRNA sequences with male liver, contains no α_{2u}-globulin mRNA sequences. The cDNA remaining single-stranded is isolated by hydroxylapatite chromatography and is shown to be specific for α_{2u}-globulin mRNA by several criteria.

Good correlation was found in all endocrine states studied between the hepatic level of α_{2u}-globulin, the level of functional α_{2u}-globulin mRNA as assayed in a wheat germ cell-free translational system, and the level of α_{2u}-globulin mRNA sequences as measured by hybridization to the α_{2u}-globulin cDNA. Thus, the hormonal control of hepatic α_{2u}-globulin synthesis by sex steroids and thyroid hormone occurs through modulation of the cellular level of α_{2u}-globulin mRNA sequences, presumably by hormonal control of transcriptive synthesis.

Our laboratory has focused on the biochemical processes underlying the multihormonal control of the synthesis of the male rat hepatic protein, α_{2u}-globulin. This protein is present in the urine of mature male rats and absent from the urine of female rats (1). It is synthesized in the liver, representing approximately 1% of hepatic protein synthesis (2), is secreted into the serum, and is excreted in the urine. Its function is uncertain. The hepatic synthesis of α_{2u}-globulin is under multihormonal control: androgen, glucocorticoid, thyroid hormone, and pituitary growth hormone are necessary for a normal rate of α_{2u}-globulin synthesis, and estrogens repress the synthesis of this protein (3–5). We have shown that hormonal modulation of the rate of hepatic α_{2u}-globulin synthesis occurs via modulation of the level of functional α_{2u}-globulin mRNA, as assayed in a cell-free translational system (3–5). Several hepatomas that produce no α_{2u}-globulin also lack translatable mRNA for this protein (2). However, these studies provide no information as to whether the hormonal regulation of translatable α_{2u}-globulin mRNA is due to transcriptional control, or to modulation at the levels of RNA processing and/or transport, or, in the case of the hepatomas, to a gene deletion. Distinguishing between these alternatives requires a pure complementary DNA (cDNA) specific for α_{2u}-globulin mRNA. This labeled cDNA can then be used to measure genomic, nuclear, and cytoplasmic α_{2u}-globulin sequences.

We have developed a procedure for the preparation of a pure α_{2u}-globulin cDNA, involving the following steps: (*i*) Partial purification of α_{2u}-globulin-synthesizing polysomes by incubation of male rat liver polysomes with rabbit antiserum to α_{2u}-globulin followed by incubation with cellulose-bound goat antiserum to rabbit IgG (6). (*ii*) Isolation of poly(A)-containing mRNA from these α_{2u}-globulin-enriched polysomes and synthesis of an α_{2u}-globulin-enriched cDNA by using reverse transcriptase (RNA-dependent DNA polymerase). (*iii*) Removal of non-α_{2u}-globulin cDNA species by hybridization of this enriched cDNA to a R_0t [RNA concentration (moles of nucleotide per liter) × incubation time (sec)] of 1000 with female liver mRNA, which contains no α_{2u}-globulin mRNA. The cDNA remaining unhybridized to female mRNA is isolated with hydroxylapatite. (*iv*) Hybridization of this single-stranded cDNA with male liver mRNA to a R_0t of 10 and isolation of the hybridized cDNA, to eliminate the short nonhybridizable cDNA strands. We have characterized the resulting "α_{2u}-globulin cDNA" to show that it is indeed specific for α_{2u}-globulin sequences.

This cDNA probe was used in RNA-driven hybridization reactions with hepatic poly(A)-containing mRNA derived from rats in various endocrine and developmental states, to measure the level of α_{2u}-globulin mRNA sequences produced in response to various hormonal manipulations. It was found, in all instances, that the level of α_{2u}-globulin mRNA sequences paralleled the translationally functional level of α_{2u}-globulin mRNA as assayed in a cell-free translational system, which in turn correlated with the rate of synthesis of the protein *in vivo*. The findings support the hypothesis that the hormonal induction of α_{2u}-globulin involves specific effects upon gene transcription.

MATERIALS AND METHODS

Purification of α_{2u}-Globulin and Rabbit Anti-α_{2u}-Globulin. α_{2u}-Globulin was purified to homogeneity from male rat urine as described previously (4). Rabbit anti-α_{2u}-globulin was generated by 4 weekly intracutaneous injections of antigen (200 μg/kg) in Freund's complete adjuvant. To isolate pure anti-α_{2u}-globulin, immune rabbit serum, in phosphate-buffered saline (PBS), was passed over a CNBr-activated Sepharose column to which pure α_{2u}-globulin had been coupled. The

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Abbreviations: cDNA, complementary DNA; PBS, 10 mM sodium phosphate, pH 7.2/0.14 M NaCl; R_0t, moles of ribonucleotide/liter × time in seconds; $R_0t_{1/2}$, R_0t value for half-maximal hybridization to cDNA; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate; PAB-cellulose, *p*-aminobenzylcellulose.

column was washed thoroughly with PBS and the pure anti-α_{2u}-globulin was eluted with 3 M NaSCN/10 mM sodium phosphate, pH 7.2. Fractions with 0.2 A_{280} unit/ml were collected and dialyzed for three 12-hr periods against 10 mM sodium phosphate at pH 7.2 containing, successively, 1.0 M, 0.5 M, and 0.14 M NaCl (An *A* unit is the amount of material having an absorbance of 1 at the specified wavelength when dissolved in 1 ml and the light path is 1 cm.). Rabbit anti-α_{2u}-globulin prepared in this manner was free of RNase activity (6). Where indicated, anti-α_{2u}-globulin was iodinated using Bolton–Hunter Reagent (New England Nuclear) (7).

Preparation of Polysomes. Total rat liver polysomes were prepared by using a modification of the procedure described by Ramsey and Steele (8). Rat liver was homogenized in 3 volumes of 50 mM *N*-2-hydroxyethyl-*N'*-2-ethanesulfonate (Hepes) pH 7.6/0.3 M KCl/5 mM magnesium acetate/3 mM glutathione and 0.25 M sucrose, using a Teflon–glass homogenizer. Triton X-100 was then added to a final concentration of 1% (vol/vol). The mixture was rehomogenized and centrifuged at 1500 × *g* for 5 min. to remove nuclei. The supernatant was collected, and 1/9 volume of 13% (wt/wt) sodium deoxycholate was added. The mixture was homogenized briefly, layered on a discontinuous sucrose gradient consisting of 5 ml of 2.0 M sucrose and 5 ml of 1.4 M sucrose in homogenization buffer and centrifuged for 20 hr at 300,000 × *g*. The sucrose layers were removed, and the surface of the polysome pellet was washed lightly with 25 mM Hepes, pH 7.6/25 mM NaCl/5 mM magnesium acetate (polysome buffer). After resuspension in polysome buffer containing heparin at 0.5 mg/ml, the polysomes were dispersed with a Teflon–glass homogenizer with a loose-fitting pestle and centrifuged at 10,000 × *g* for 5 min to remove aggregated material. The polysomes (75–100 A_{260} units/ml) were frozen in liquid nitrogen and stored at −90°.

Binding of ^{125}I-labeled Anti-α_{2u}-Globulin to Rat Liver Polysomes. Rat liver polysomes (10–20 A_{260} units/ml) were incubated for 2 hr at 0° with various amounts of ^{125}I-labeled anti-α_{2u}-globulin in polysome buffer containing 0.2 M NaCl, heparin at 0.5 mg/ml, and 0.5% Triton X-100. The mixture was then passed through a column (1 cm × 40 cm) of Ultrogel AcA 34 (LKB Co.) in the same buffer at 4°. Fractions were collected and the A_{260} and ^{125}I cpm were monitored. Polysomes (along with any bound IgG) pass through this column in the void volume, while unbound IgG is retarded (exclusion limit = 400,000 daltons). Where indicated, the polysomes in the void volume were fractionated on linear 15–50% sucrose gradients in polysome buffer at 120,000 × *g* for 3 hr. The gradients were collected from the bottom and A_{260} and ^{125}I cpm were monitored. With this procedure, it was found that maximum binding of rabbit anti-α_{2u}-globulin IgG to male rat liver polysomes occurred at 1.5–2 μg of IgG per A_{260} unit of polysomes.

Preparation of *P*-Aminobenzylcellulose–Goat Anti-Rabbit Matrix. Goat antiserum to rabbit IgG (a kind gift from F. Margolis, Roche Institute for Molecular Biology) was passed through a rabbit IgG-Sepharose column in PBS (6). The column was washed and the goat anti-rabbit IgG was eluted and dialyzed as described (6). *p*-Aminobenzylcellulose (PAB-cellulose; Servacel pab 23; Accurate Chemicals) was pretreated as described and linked to the goat anti-rabbit IgG via diazotization (6).

Partial Purification of α_{2u}-Globulin mRNA. Rat liver polysomes (10,000–15,000 A_{260} units) in 200 ml of polysome buffer containing 0.2 M NaCl were incubated with rabbit anti-α_{2u}-globulin (2 μg of IgG per A_{260} unit of polysomes) for 2 hr at 0° with gentle swirling. The polysomes, containing bound antibody, were separated from unbound antibody by chromatography through a large column (10 g) of streptococcal protein A-Sepharose (Pharmacia) at 4° in polysome buffer. The porosity of Sepharose is such that polysome-bound IgG cannot interact efficiently with the immobilized protein A, while free IgG is retained efficiently. The polysome–anti-α_{2u}-globulin complex was then mixed batchwise with the PAB-cellulose–goat anti-rabbit matrix (1 g of PAB-cellulose, containing 175–200 mg of IgG), and the cellulose suspension was dispersed, swirled for 2 hr at 0°, and centrifuged for 10 min at 4000 × *g*. The supernatant was removed and the cellulose was dispersed and washed five times with polysome buffer. The bound polysomes were then dissociated by stirring the cellulose in 50 ml of 10 mM Hepes, pH 7.6/20 mM EDTA for 15 min. The cellulose was removed by centrifugation and the supernatant was collected. The procedure was repeated with an additional 50 ml of EDTA buffer and the supernatants were combined, brought to 0.5 M NaCl, 0.5% sodium dodecyl sulfate, and passed through oligo(dT)-cellulose to obtain the poly(A)-containing RNA as described by Krystosek *et al.* (9).

Cell-Free Protein Synthesis. An mRNA-dependent wheat germ cell-free translational system was prepared as outlined previously (4, 5) with the modifications described by Jones *et al.* (10). mRNA-dependent protein synthesis and analysis of total proteins and α_{2u}-globulin synthesized *in vitro* were performed as described (2, 4, 5).

Preparation of cDNA. cDNA was synthesized from poly(A)-containing RNA using [^{3}H]dCTP (24 Ci/mmol) and reverse transcriptase of avian myeloblastosis virus (obtained from J. W. Beard) as described by Kacian and Myers (11). RNA-excess annealing reactions were done at 69° in 0.4 M NaCl under paraffin oil as described by Axel *et al.* (12). Hybridization was monitored with S1 nuclease (Miles). For the purification of α_{2u}-globulin cDNA, a [^{3}H]cDNA was synthesized to the α_{2u}-globulin-enriched mRNA preparation obtained using the PAB-cellulose technique. This cDNA was then hybridized with female rat liver mRNA in a final volume of 200 μl to a R_0t of 1000. The hybridization mixture was diluted with 10 volumes of 0.01 M sodium phosphate buffer, pH 6.85, and passed through 0.5 g of hydroxylapatite (Bio-Rad) at 65°. Single-stranded cDNA, containing the α_{2u}-globulin cDNA, was eluted with 0.12 M sodium phosphate buffer, pH 6.85, and hybrids were eluted with 0.4 M buffer. The single-stranded cDNA was brought to 0.5 M NaOH and left overnight at room temperature. The solution was neutralized and desalted over a Sephadex G-50 column equilibrated with 10 mM Tris, pH 7.5/0.15 M NaCl/2 mM EDTA/0.1% sodium dodecyl sulfate. Void volume fractions containing radioactivity were pooled and precipitated at −20° with ethanol and *Escherichia coli* carrier DNA. The single-stranded cDNA prepared by this procedure contains the α_{2u}-globulin cDNA, but is also enriched for short, nonhybridizable cDNAs. To remove these species, this cDNA was annealed to male liver mRNA to a R_0t of 10, and the hybridization mixture was diluted with 40 volumes of S1 nuclease buffer (12). Denatured *E. coli* DNA was added to 10 μg/ml, followed by S1 nuclease (1000 units/ml). The mixture was incubated for 45 min at 37°, and was then boiled for 5 min in 0.5 M NaOH (13). The solution was neutralized and passed over a Sephadex G-50 column as outlined above. The cDNA in the void volume was collected and precipitated with ethanol. cDNA prepared in this manner could hybridize to 65–75% with male liver mRNA.

RESULTS

The partial purification of α_{2u}-globulin polysomes by means of this double-antibody technique requires the specific binding of rabbit anti-α_{2u}-globulin to the nascent α_{2u}-globulin chains.

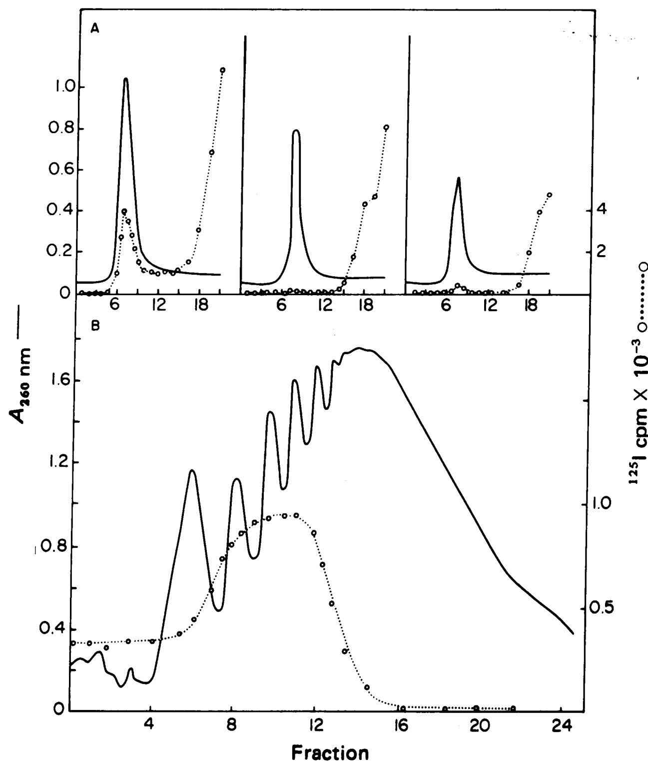

FIG. 1. Binding of ^{125}I-labeled anti-α_{2u}-globulin to rat liver polysomes. ^{125}I-Labeled anti-α_{2u}-globulin was incubated with liver polysomes and passed through a column of Ultrogel AcA34, and the A_{260} and ^{125}I cpm were monitored. (*A*) Labeled antibody binding to: (*Left*) male liver polysomes; (*Center*) female liver polysomes; (*Right*) male polysomes preincubated with unlabeled anti-α_{2u}-globulin (10 μg of IgG per A_{260} unit of polysomes). (*B*) the polysomes in the void volume of the Ultrogel column shown in *A left* were fractionated on a 15–50% sucrose gradient, and the A_{260} and ^{125}I cpm were monitored.

This binding was monitored by incubating ^{125}I-labeled antibody with male and female rat liver polysomes, followed by chromatography on Ultrogel AcA 34. Labeled anti-α_{2u}-globulin was found to bind to male rat liver polysomes (Fig. 1), but not to female liver polysomes or to male polysomes previously incubated with an excess of unlabeled anti-α_{2u}-globulin (Fig. 1*A*).

On a preparative scale, approximately 10,000 A_{260} units of polysomes was incubated with rabbit anti-α_{2u}-globulin, followed by cellulose-bound goat anti-rabbit IgG, as outlined in *Materials and Methods*. The polysomes that remained bound to the cellulose after several washes were dissociated and eluted with EDTA, and the poly(A)-containing mRNA was isolated and translated. Fig. 2*A* shows the pattern of total proteins synthesized *in vitro* by this mRNA fraction. A prominent peak is seen that migrates with authentic α_{2u}-globulin on sodium dodecyl sulfate/polyacrylamide gels. No such peak is discernible in the pattern of protein synthesis directed by total male liver mRNA (Fig. 2*B*). That this peak is indeed α_{2u}-globulin is shown by immunoprecipitation with anti-α_{2u}-globulin and gel electrophoresis of the solubilized immunoprecipitate (Fig. 2*C*).

A precise estimate of the purity of a given mRNA cannot be obtained in a cell-free translational system, because these systems translate smaller mRNAs more efficiently than larger ones. The α_{2u}-globulin-enriched mRNA sample represented in Fig. 2*A* appears to be 30–35% α_{2u}-globulin mRNA, as evidenced by the hybridization of its corresponding cDNA to female liver

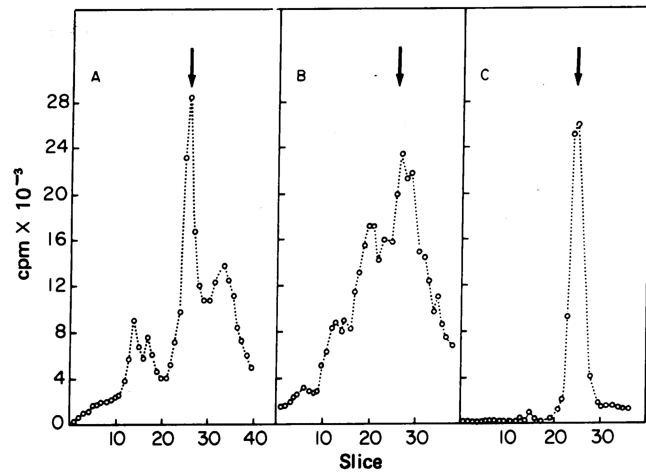

FIG. 2. Sodium dodecyl sulfate/polyacrylamide gel electrophoretic profile of proteins synthesized *in vitro* by the α_{2u}-globulin-enriched mRNA. (*A*) Total released proteins synthesized in the wheat germ system in response to 5 μg of α_{2u}-globulin-enriched mRNA. (*B*) Total released proteins synthesized in response to 5 μg of unfractionated male liver mRNA. (*C*) Immunoprecipitated α_{2u}-globulin synthesized *in vitro* by 5 μg of the α_{2u}-globulin-enriched mRNA. Arrows mark positions of authentic α_{2u}-globulin.

mRNA (see below). This PAB-cellulose technique typically yielded 40–70 μg of mRNA, which contained 20–50% α_{2u}-globulin mRNA, from 10,000 A_{260} units of starting polysomes.

Advantage was taken of the fact that female liver contains no detectable α_{2u}-globulin mRNA, enabling purification of α_{2u}-globulin cDNA by preparative hybridization. A cDNA was made to the α_{2u}-globulin-enriched mRNA, and annealed to a high R_0t with female mRNA. The fraction remaining single-stranded was collected by means of hydroxylapatite. This cDNA was then hybridized with male liver mRNA to a R_0t of 10 to remove the short, nonhybridizable strands. The resulting cDNA is now found to be specific for α_{2u}-globulin mRNA. It hybridizes with a single transition to male liver mRNA (Fig. 3) with a $R_0t_{1/2}$ of 0.2, which indicates that the driver mRNA species represents approximately 1% of the total mRNA population. This cDNA does not hybridize, by a R_0t of 400, to female liver mRNA or to mRNA from male kidney, brain, or spleen (Fig. 3*A*). This cDNA hybridizes to the α_{2u}-globulin-enriched mRNA with a $R_0t_{1/2}$ of 0.006, indicating that the hybridizing mRNA species is 33-fold purified in this enriched mRNA sample, relative to male liver mRNA. Functional α_{2u}-globulin mRNA can be induced in ovariectomized females by treatment with androgens (3). As seen in Fig. 3*A*, the α_{2u}-globulin cDNA hybridizes to liver mRNA from an androgen-treated ovariectomized female, with a $R_0t_{1/2}$ which indicates that the level of α_{2u}-globulin sequences in this mRNA sample is 10% of that in a normal male, the level which corresponds to that found translationally (Table 1). Our cDNA is thus specific for an mRNA sequence that is not present in normal female liver, but which can be induced in females by ovariectomy and androgen treatment, characteristics expected for α_{2u}-globulin cDNA.

If a fixed amount of this cDNA is titrated with increasing amounts of male mRNA in a cDNA-excess hybridization reaction, the initial slope, extrapolated to 100% hybridization, indicates that 0.1 ng of this cDNA would be completely protected from nuclease digestion by approximately 11.5 ng of male liver mRNA (Fig. 4*A*), again indicating that the hybridizing mRNA species represents approximately 1% of the total mRNA.

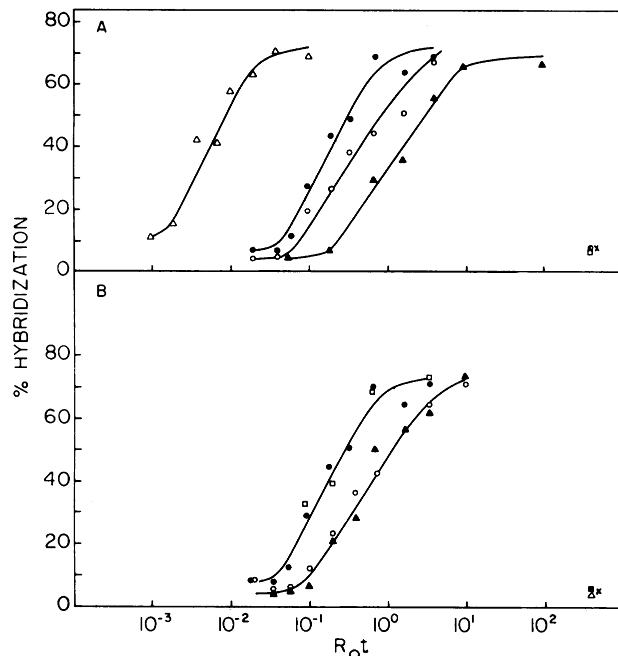

FIG. 3. Hybridization of hepatic mRNAs to labeled α_{2u}-globulin cDNA. (*A*) Hybridization to: ●, male liver mRNA; ◐, female liver mRNA; □, kidney mRNA; ×, spleen mRNA; ○, castrated male liver mRNA; ▲, liver mRNA from an ovariectomized female treated with androgens; △, α_{2u}-globulin-enriched mRNA. (*B*) Hybridization to: ●, male liver mRNA, ■, thyroidectomized male liver mRNA; □, liver mRNA from a thyroidectomized male treated with thyroxine for 10 days: △, liver mRNA from a prepubescent 20-day-old male; ▲, liver mRNA from a 45-day-old male; ○, liver mRNA from an adult male treated with estradiol-17β for 4 days; ×, brain mRNA.

To further demonstrate the purity of this cDNA probe, male liver mRNA was subjected to sucrose gradient centrifugation, and the gradient fractions were assayed in the wheat germ system for α_{2u}-globulin-synthesizing activity. The translational activity was found in a rather broad peak sedimenting between

Table 1. α_{2u}-Globulin translational activity and kinetics of hybridization

mRNA	Translational activity*	$R_0t_{1/2}$†	$20\ (R_0t_{1/2})^{-1}$
Adult male liver	100	0.2	100
Adult female liver	0	>400	<5
Ovariectomized female + androgens	12	1.5	13
Male kidney, spleen, brain	0	>400	<5
Thyroidectomized male	0	>400	<5
Thyroidectomized male + thyroxine	110	0.2	100
Castrated male	30	0.4	50
Adult male + estradiol-17β	45	0.6	33
20-day-old male	0	>400	<5
45-day-old male	35	0.6	33
α_{2u}-Globulin-enriched mRNA	3000	0.006	3300

* α_{2u}-Globulin synthesis *in vitro* in the wheat germ system was determined as described in *Materials and Methods.* Values given are percent of adult male.

† $R_0t_{1/2}$ determined from the hybridization curves shown in Fig. 3.

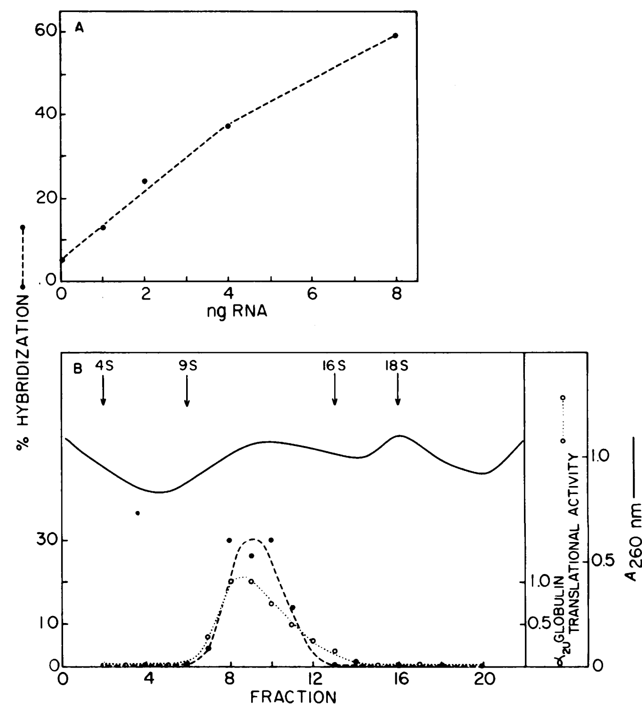

FIG. 4. cDNA-excess hybridization of α_{2u}-globulin cDNA to total and fractionated male liver mRNA. (*A*) 0.1 ng of [^{3}H]cDNA (~1500 cpm) was incubated with increasing amounts of male liver mRNA at 50° for 70 hr in 20 μl of 70% (vol/vol) formamide/0.4 M NaCl/0.01 M piperazine-*N*,*N'*-bis(2-ethanesulfonate) (Pipes) (pH 6.4)/0.01% sodium dodecyl sulfate/yeast RNA at 200 μg/ml (14). Hybridization was monitored with S1 nuclease (12). (*B*) Male liver mRNA was denatured with dimethyl sulfoxide and fractionated on linear 15–30% sucrose gradients containing sodium dodecyl sulfate (15). Fractions were collected and assayed for α_{2u}-globulin translational activity in the wheat germ system. Four ng of RNA from each gradient fraction was also incubated with 0.1 ng of the α_{2u}-globulin cDNA as described in *A*. Hybridization was monitored with S1 nuclease.

10 and 15 S (Fig. 4*B*). The gradient mRNA fractions were then used for cDNA-excess hybridization with the α_{2u}-globulin cDNA. Hybridization was found only with those fractions that contained α_{2u}-globulin translational activity (Fig. 4*B*). This correlation between translational activity and hybridization and the absence of hybridization elsewhere in the gradient indicate that the cDNA is specific for α_{2u}-globulin mRNA sequences.

This α_{2u}-globulin cDNA was then used to measure hepatic α_{2u}-globulin mRNA sequences in rats in various hormonal and developmental states. Hepatic poly(A)-containing mRNA was extracted from these animals, the α_{2u}-globulin translational activity of each mRNA sample was assayed in the wheat germ system, and the level of α_{2u}-globulin mRNA sequences was quantitated with the cDNA probe. In each case of hormonal modulation it was found that the level of hybridizable α_{2u}-globulin mRNA sequences correlated well with the α_{2u}-globulin translational activity of the mRNA sample (Fig. 3, Table 1), which in turn paralleled the tissue level of the protein. Hepatic mRNA samples that contained no detectable translatable α_{2u}-globulin mRNA (thyroidectomized male and 20-day-old male) showed no hybridization to the α_{2u}-globulin cDNA by a R_0t of 400, indicating that there is less than one copy of α_{2u}-globulin mRNA per cell in these livers (Fig. 3). Hepatic messenger RNA samples from rats in various endocrine deficiency states, which contained diminished α_{2u}-globulin translational activity relative to male mRNA, hybridized to the cDNA probe with correspondingly higher $R_0t_{1/2}$'s than that

observed for male mRNA (Fig. 3). Hepatic mRNA from thyroidectomized males treated for 10 days with thyroxine, in which the α_{2u}-globulin translational activity has been induced to a normal level, hybridized to the α_{2u}-globulin cDNA with kinetics indistinguishable from those observed for male liver mRNA (Fig. 3*B*).

DISCUSSION

Studies on hormonal control of the biosynthesis of secretory and viral proteins have been greatly facilitated by the availability of cDNA probes for the messenger RNAs coding for these proteins (16–20). We have developed a method for the preparation of a complementary DNA specific for the mRNA coding for α_{2u}-globulin, a minor rat liver protein under multihormonal control.

This cDNA probe was used to quantitate hepatic α_{2u}-globulin mRNA sequences in rats in various endocrine states. It was found that, under all endocrine manipulations, the rate of hepatic α_{2u}-globulin biosynthesis parallels the level of hepatic α_{2u}-globulin mRNA sequences. Our previous findings (3–5), which indicated that the hormonal modulation of α_{2u}-globulin biosynthesis occurs through modulation of the level of translationally functional mRNA, are now extended to demonstrate that this modulation is not the result of activation of nontranslatable α_{2u}-globulin mRNA sequences in response to hormones. This correlation between the rate of synthesis of α_{2u}-globulin and the level of its corresponding mRNA indicates that translational control is not a major factor in the hormonal modulation of the synthesis of this protein. These findings are consistent with control at the level of transcription, and are in accord with the models of hormonal control that have been suggested in several other systems (17–22).

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