Post-transcriptional regulation of rat liver gene expression by glucocorticoids

Ruth Fulton and George D. Birnie

The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, UK, and

John T. Knowler

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, UK

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SUMMARY

We have investigated the mechanisms whereby glucocorticoids control the expression of specific genes in the livers of adult male rats. Construction and differential screening of a cDNA library representing normal rat liver polysomal poly(A)⁺ RNAs allowed selection of probes for hormonally regulated genes. The mechanism of this regulation was analysed by studying the changes in relative abundance of the RNA sequences homologous to four selected recombinants in RNA from subcellular fractions of liver, and comparing them with that of albumin mRNA. The relative abundance of these four RNA sequences increased to varying degrees in the nucleus, whilst that of three of them was concomitantly depleted in polysomal RNA when circulating levels of glucocorticoids were negligible, i.e. 14 days after adrenalectomy. One of the sequences was identified as $\alpha_2\mu$ -globulin mRNA. Within 2 hours of injecting Dexamethasone (a synthetic glucocorticoid) into rats that had been adrenalectomised 14 days previously, the relative abundances of $\alpha_2\mu$ -globulin RNA in nuclear and polysomal RNA returned to those found in normal rat liver. The data indicate that reduced glucocorticoid levels lead to sequence specific retention of RNA in the nucleus and that the RNA retained is released to the cytoplasm following glucocorticoid injection. Our results provide an example, for the first time, of glucocorticoid regulation of gene expression at the post-transcriptional level of nucleo-cytoplasmic transport.

INTRODUCTION

Glucocorticoids enhance the expression of genes encoding several liver gluconeogenic enzymes, for example tyrosine amino transferase (1) and tryptophan oxygenase (2). They also have a generally hypertrophic effect, stimulating ribosomal RNA synthesis by three-fold (3). This regulatory control of gene expression by glucocorticoids has been shown to occur at the level of transcription, in agreement with the generally accepted mechanism of steroid hormone action (4). However, evidence is accumulating that hormones may also play a significant role in post-transcriptional regulation of gene expression: oestrogen can enhance the cytoplasmic stability of vitellogenin mRNA possibly by specifically binding to the mRNA (5), and the stability of growth hormone mRNA is enhanced 50-fold by the glucocorticoid analogue

Dexamethasone in pituitary tumour cells (6). Moreover, Schumm and Webb (7) have shown that hormonal regulation of the release of nucleus-confined RNA molecules can occur.

To study the effect of glucocorticoids on gene expression in rat liver we made comparisons between normal rat liver mRNA abundances and corresponding mRNA abundances in the livers of animals from which the adrenal glands had been removed 14 days previously (8). Removal of the adrenal glands results in a 500-fold reduction in the original peripheral blood glucocorticoid levels producing an animal upon which the effect of a virtual absence of the hormone can be investigated and to which known quantities of glucocorticoids can be subsequently administered. The concomitant loss of mineralocorticoid and catecholamine production in adrenalectomised animals is compensated for by provision of saline in place of water and maintenance of the animals in an environment in which stress is minimised.

Bajwa *et al.* (8) investigated the effects of adrenalectomy on the complexity and relative abundances of polyadenylated mRNAs in rat liver. These analyses indicated that, by 14 days after adrenalectomy, about 7,000 sequences could no longer be detected in liver polysomes, but that some sequences increased in abundance. In order to study the mechanism of glucocorticoid regulation of gene expression in rat liver in more detail, our approach has involved differential screening of a normal rat liver cDNA library with cDNAs transcribed from polysomal poly(A)⁺ RNA from normal rat liver and from the livers of adrenalectomised rats, to allow selection of hormonally regulated sequences. This method of comparative cross-screening of a cDNA library with both a homologous and a heterologous probe has been employed successfully by Scherer *et al.* (1) to isolate cDNA clones coding for rat tyrosine aminotransferase, and also in cloning cDNA sequences of hormone-regulated genes from the MCF-7 breast cancer cell line (9).

The results presented in this paper, especially those for ZE10 (identified as encoding a 3' portion of an $\alpha_2\mu$ -globulin mRNA), demonstrate regulation of gene expression at the post-transcriptional level of nucleocytoplasmic transport.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200–250g were used in all experiments. The animals were kept in temperature-controlled rooms under 12-hour alternating light-dark cycles and fed *ad libitum*. Bilateral adrenalectomy was performed

via a dorsal approach under ether anaesthesia and adrenalectomised animals were given 0.9% (w/v) NaCl in place of water.

Blood samples were taken prior to sacrifice in order to assess the effectiveness of adrenalectomy in depleting circulating glucocorticoids. Anticoagulated blood samples were centrifuged at $3,000g_{av}$ for 5 min and the supernatants were analysed for corticosteroids by Dr. R. Frazer of the Blood Pressure Unit of Glasgow Western Infirmary. RNA was only prepared from the livers of animals which had negligible ($<1 \times 10^{-11}M$) blood glucocorticoid levels 14 days after adrenalectomy.

Dexamethasone sodium phosphate succinate, a generous gift from Merck, Sharpe and Dohme Ltd., was dissolved at 2 mg/ml in 0.9% (w/v) NaCl. Animals were injected intraperitoneally with 1 mg/100g body weight and sacrificed 2 h later.

Isolation of RNA

RNA was prepared from whole liver and from sub-cellular fractions of liver (nuclei, cytosol and polysomes). The liver was collected in ice-cold phosphate-buffered saline then finely chopped and hand homogenised in a 0.25M sucrose buffer containing 25 mM KCl, 3 mM $MgCl_2$ and 50 mM tris-HCl, pH 7.6. Nuclei and cell debris were pelleted ($1000g_{max}$ for 10 min) and the supernatant layered over a 2M sucrose pad after α -amylase and Triton X-100 treatment (10). Centrifugation at $170,000g_{max}$ for 3 h pelleted the polysomes, from which the RNA was extracted by a LiCl/urea precipitation method as previously described (11). An equal volume of 4M LiCl/8M urea was added to the supernatant to precipitate cytosolic RNA.

Purified nuclei were prepared from the crude pellet of nuclei and cell debris by the citric acid method of Busch and Smetana (12). Preparation of RNA from total cells or nuclei involved lysing them in a solution of 5M guanidinium thiocyanate which was then layered over a pad of 5.7M CsCl as described by Chirgwin *et al.* (13). Centrifugation at $210,000g_{max}$ for 40 h pelleted the nuclear and total RNA.

The polyadenylated fraction of each RNA was isolated using oligo(dT)-cellulose chromatography as described by Aviv and Leder (14).

Preparation and screening of cDNA library

Normal rat liver polysomal poly(A)⁺ RNA was reverse-transcribed using an oligo(dT)₁₇ primer providing a single-stranded cDNA with a 5' hairpin loop. DNA polymerase (BRL) was utilised to produce double-stranded cDNA which was blunt-ended using S1-nuclease and ligated into the flush-ended and phosphatased BamHI site of pAT153. Transformation of *E.coli* HB101 allowed

selection of recombinants which were Amp^RTet^S; 350 of these recombinants were individually picked and inoculated into 96-well microtitre dishes (15). With the help of transfer plates (Dynatech) duplicate impressions were taken of the clones in the microtitre dishes and these were grown overnight on Sartorius nitrocellulose on L-agar ampicillin plates. They were prepared for hybridisation as described by Thayer (16).

Plasmid preparation

Small-scale plasmid preparations from 5 ml cultures were carried out using the alkaline extraction procedure of Birnboim and Doly (17). Bulk plasmid preparations were made from 500 ml cultures using the same method and including a final purification step of CsCl-ethidium bromide centrifugation (18).

Northern blotting and RNA dotting

Denaturation and electrophoretic separation of RNA on 1% (w/v) agarose gels containing formaldehyde, and transfer of RNA to Sartorius nitrocellulose, were done as described by Thomas (19). In the RNA doubling-dilution dotting method, 0.2 µg of poly(A)⁺ RNA in 4 µl was dotted in duplicate on the first position on the nitrocellulose followed by 0.1 µg on the next position, and so on (20). A series of dilutions from a single preparation of poly(A)⁺ RNA from normal whole rat liver was included as a standard on each membrane. The membranes were then baked for 2 h at 80°C. Hybridisation was done essentially as described by Wahl *et al.* (21) and membranes were washed at room temperature in 3xSSC followed by 0.5xSSC then 0.1xSSC with all buffers preheated to 65°C. Kodak X-Omat film was exposed to the filters at -70°C with Dupont Lightning Plus intensifying screens for an appropriate length of time.

Preparation of probes

RNA was reverse-transcribed as described by Birnie *et al.* (22) using $\alpha^{32}\text{P}$ -dCTP (410 Ci/mmol; Amersham International). Plasmid DNAs were labelled by nick-translation using an Amersham International kit and $\alpha^{32}\text{P}$ -dCTP (410 Ci/mmol).

DNA sequencing strategy and method

ZE10 recombinant DNA was digested with restriction enzymes SphI and BamHI under conditions recommended by the supplier (Bethesda Research Laboratories). The M13 vectors Mp18 and Mp19 (23) were also digested with BamHI and SphI and all digested fragments were eluted from low melting-point agarose gels. T4 DNA ligase (BRL) was used to ligate the 670 bp ZE10 fragment into the Mp18 and Mp19 vectors and several clones of each were

sequenced by the Sanger dideoxy chain termination method (24) in the presence of $\alpha^{35}\text{S}$ -dATP according to the strategy outlined in Figure 3.

RESULTS

Selection of probes for differentially expressed genes

A recombinant cDNA library, which represented poly(A)⁺ RNA sequences on the polysomes of Wistar rat liver, was constructed essentially as described by Wiedemann *et al.* (25). The library comprised 350 recombinants; all were picked and cultured in the wells of microtitre dishes. The colonies were then grown in ordered arrays on nitrocellulose membranes and prepared for colony hybridisation as described by Thayer (16). Duplicate membranes were hybridised with ³²P-cDNA reverse transcribed from polysomal poly(A)⁺ RNA from either normal rat liver or liver from rats that had been adrenalectomised 14 days previously, and the membranes autoradiographed. Since the intensity of the signal from each colony is approximately proportional to the concentration of the corresponding cDNA in the ³²P-cDNA (26), comparison of the signals from corresponding colonies allowed us to identify tentatively recombinants homologous to polysomal poly(A)⁺ RNA sequences that changed in relative abundance following adrenalectomy. Of the 350 colonies so screened, about 60% were detectable as above background when hybridised with ³²P-cDNA from normal rat liver polysomal poly(A)⁺ RNA. None of the recombinants consistently showed an increased hybridisation signal when screened with ³²P-cDNA from polysomal poly(A)⁺ RNA from the liver of an adrenalectomised rat; however, at least 10 showed a decreased signal, indicating that these may represent polysomal mRNAs that are depleted in relative abundance following adrenalectomy.

Because the reproducibility of colony hybridisation data depends on a number of factors that are not easily controlled, 20 recombinants, including all which showed a decreased signal in the colony hybridisation assay, were re-screened using the DNA dot hybridisation method of Thomas (19). Approximately 100 ng of each recombinant plasmid DNA was fixed on duplicate nitrocellulose membranes which were hybridised to ³²P-cDNA transcribed from liver polysomal poly(A)⁺ RNA from normal and adrenalectomised rats. At least 4 of the 20 recombinants selected were found to represent mRNAs that appeared to be depleted significantly on liver polysomes 14 days after adrenalectomy (data not shown), and so were chosen for further study.

The sizes of the cDNA inserts in these four recombinants, determined by restriction enzyme digestion and agarose gel electrophoresis, ranged from 250

TABLE 1: Insert and transcript sizes of selected recombinants

Recombinant	Insert size (bp)	Transcript size (nucleotides)
ZE10	370	1120
XE5	505	1500
YE5	280	724
XE2	250	1530

to 505 base pairs (Table 1). The sizes of their homologous RNAs, determined by Northern blot analysis of electrophoretically fractionated total poly(A)⁺ RNA from normal rat liver, ranged from 724 to 1530 nucleotides (Table 1).

Relative abundances of selected RNAs in liver from normal and adrenalectomised rats

Nuclear, polysomal and cytosolic fractions were prepared from livers from normal rats and from rats 14 days after adrenalectomy. Poly(A)⁺ RNA was isolated from these fractions, and from whole liver RNA from normal and adrenalectomised rats, and the relative abundances of the RNAs homologous to the 4 selected recombinants (Table 1) determined by doubling-dilution dot-blot hybridisation. Comparison of whole liver and polysomal poly(A)⁺ RNAs

Figure 1: Relative abundance of ZE10 RNA in RNA from subcellular liver fractions. Poly(A)⁺ RNA from liver cytosol, polysomes and whole tissue were dotted in a doubling dilution fashion with 0.2 µg of each RNA on the first position and probed with ³²P-labelled ZE10 DNA. (Abbreviations as for Tables 2 and 3).

TABLE 2: Mean relative abundances of five poly(A)⁺ RNAs in RNA from subcellular fractions of liver(a)

Source of RNA(b)	Recombinant Probe				Albumin
	ZE10	XE5	YE5	XE2	
Polysomes					
N P ⁺ (c)	80	133	125	100	25
Ad P ⁺	21	17	75	120	50
Nuclei					
N Nucl ⁺	20	10	6	12	3
Ad Nucl ⁺	83	133	112	100	12.5
Cytosol					
N Csol ⁺	8	8	32	8	N/d
Ad Csol ⁺	6	10	0	7	N/d

- (a) In each case the relative abundance is related to that in a single standard RNA preparation from normal whole rat liver, arbitrarily given the value 100 (see Materials and Methods).
- (b) All values are the mean of independent assays of three different RNA preparations and were done in duplicate.
- (c) N: normal; Ad: 14 days after adrenalectomy; P: polysomal; Nucl: nuclear; Csol: cytosolic; +: poly(A)⁺ RNA.

(for example, see Fig. 1) showed that the relative abundances of these RNAs were not decreased in whole liver RNA from adrenalectomised rats. However, 3 of the RNAs (YE5, ZE10 and XE5) were depleted, to varying extents (2-fold to 8-fold), in poly(A)⁺ RNA from liver polysomes after adrenalectomy (see Table 2). Moreover, there was no significant accumulation of these sequences in cytosolic poly(A)⁺ RNA, as would have been expected if the mRNAs had been retained in the liver cytoplasm (Fig.1 and Table 2).

This apparent anomaly was explained by the observation that decreases in the relative abundances of, in particular, ZE10 and XE5 RNAs in liver polysomal poly(A)⁺ RNA from adrenalectomised rats were matched by concomitant increases in their relative abundances in nuclear poly(A)⁺ RNA (Fig. 2). Similar reciprocal changes in the relative abundances of these RNAs were observed in independent experiments with RNAs prepared from 3 separate groups of adrenalectomised and normal rats, the data from which have been averaged and summarised in Table 2. These experiments also showed that YE5 RNA, which was depleted only 2-fold in the polysomal RNA from adrenalectomised rats, was increased 20-fold in the liver nuclear RNA from the same animals; in addition, XE2 RNA was increased 10-fold in liver nuclear RNA after

Figure 2: Relative abundance of ZE10, XE5, YE5 and XE2 RNAs in RNA from liver nuclei and polysomes. Poly (A)⁺ RNA from liver polysomes and nuclei were dotted in a doubling dilution fashion with 0.2 µg of each RNA on the first position and probed with the ³²P-labelled recombinant DNAs. (Abbreviations as for Table 2).

adrenalectomy though no concomitant decrease in polysomal RNA was observed (Fig. 2 and Table 2).

Northern blot analyses of RNAs (e.g. see Fig. 4) showed that the changes in RNA abundances were not due to non-specific degradation. Moreover, hybridisation artefacts were ruled out by probing dot blots of RNA, in which the sequences complementary to the selected recombinant DNAs varied in abundance, with an albumin cDNA clone (27). It has been reported that glucocorticoids do not affect the abundance of albumin mRNA in liver (28), and the variations in albumin mRNA abundance that we observed between RNAs from livers of normal and adrenalectomised rats were much less than those in the abundances of the other sequences (Table 2). Further evidence which validates our interpretations comes from the fact that the same RNAs were dotted on to five membranes in a single experiment, each of which was then probed with a different one of the five recombinants (including the albumin

cDNA recombinant). The varying extents of hybridisation with each probe cannot be explained in terms of either non-specific degradation of RNA, or of variation in the extent of contamination of poly(A)⁺ RNA with ribosomal RNA.

Identification of ZE10

The RNA homologous to recombinant ZE10 was present at high abundance in normal rat liver and showed a significant response to adrenalectomy (Table 2 and Figs. 1 and 2); this recombinant was therefore selected for further study.

Hybrid-selection and cell-free translation of ZE10 RNA yielded a peptide of M_r about 18–20 kd (data not shown). This, together with the size of the polysomal mRNA (1.1 kb) and the occurrence of the RNA as a relatively high abundance mRNA in normal rat liver, suggested several candidates for the protein encoded by ZE10 RNA. One of these was the urinary protein, α_2u -globulin. To test this, a cloned cDNA sequence corresponding to 800 bp of a mouse major urinary protein mRNA was excised from the recombinant plasmid pMUP325L (29) with restriction enzymes ClaI and SalI and hybridised with ³²P-labelled ZE10 DNA. A strong signal was seen on the autoradiograph (data not shown), indicating a very significant degree of homology between the mouse urinary protein cDNA sequence and ZE10 DNA. The identity of ZE10 was confirmed when it was found that the sequence of the cDNA insert in ZE10 was identical to 85 nucleotides of the 3' end of the coding sequence of rat α_2u -globulin and 275 nucleotides of the 3'-untranslated region (Fig. 3).

Mechanism of the effect of adrenalectomy on ZE10 RNA abundance in rat liver

To determine whether differences in the polyadenylation of ZE10 RNA could explain the changes in nuclear and polysomal concentrations of this sequence that followed adrenalectomy, poly(A)⁺ and poly(A)⁻ RNA from nuclei and polysomes from livers of normal and adrenalectomised rats were titrated with ZE10 DNA. Table 3 shows that no change could be detected in the proportion of ZE10 RNA in the poly(A)⁻ fractions. An effect of adrenalectomy on the processing of ZE10 mRNA precursor was also ruled out. Northern blot analysis of nuclear RNA from normal rat liver and from the livers of adrenalectomised rats showed that the predominant RNA species was the same in both cases, and was the same as that in polysomal poly(A)⁺ RNA from normal rat liver, viz., 1.1 kb (Fig. 4a). Even after over-exposure of the X-ray film, no higher molecular weight bands of precursors to the mRNA could be detected in the nuclear poly(A)⁺ RNA from livers of adrenalectomised rats (Fig. 4b).

The effect of an exogenous glucocorticoid (Dexamethasone) on the

Figure 3: Cloning and sequencing strategy. Restriction endonuclease cleavage map of a fragment of the ZE10 recombinant; vertical lines and letters refer to the following restriction sites: EcoRI-E; Bam HI-B; SphI-Sp; Sal I-S. The nucleotide sequencing strategy is represented by horizontal arrows which indicate the extent and polarity of sequence data obtained. Above this is a diagram of the full length α_{2u} -globulin cDNA (34); the filled box represents the coding sequence and the hatched box the 3' untranslated sequence including the poly(A) tail. As indicated on the diagram ZE10 includes 85 nucleotides of the 3' coding sequence and 275 nucleotides of the 3' untranslated sequence of α_{2u} -globulin.

TABLE 3: Relative abundance of ZE10 RNA in polyadenylated and nonpolyadenylated fractions of RNA

Source of RNA(a)	Relative abundance of ZE10 RNA
N P ⁺ (b)	100
N P ⁻	0.8
Ad P ⁺	25
Ad P ⁻	<0.8
N Nucl ⁺	6
N Nucl ⁻	<0.8
Ad Nucl ⁺	25
Ad Nucl ⁻	<0.8
N Tot ⁺	100

- (a) RNAs were prepared from livers of one group of adrenalectomised rats and one group of normal rats.
- (b) N: normal; Ad: 14 days after adrenalectomy; P: polysomal; Nucl: nuclear; Tot: total cellular; +: poly(A)⁺ RNA; -: poly(A)⁻ RNA.

Figure 4: Northern blot analysis of ZE10 RNA. Poly(A)⁺ nuclear RNA from normal rat liver and from liver from a rat adrenalectomised 14 days previously were electrophoretically fractionated on a 1% agarose gel, then blotted onto nitrocellulose and probed with ³²P-labelled ZE10 DNA. The blot was autoradiographed for (a), 3 days and (b), 10 days. The molecular weight markers (left margin) were rat and *E.coli* rRNAs. (Abbreviations as for Table 2).

subcellular distribution of ZE10 RNA sequences in livers of adrenalectomised rats was determined by measuring the relative abundances of ZE10 RNA in nuclear and polysomal poly(A)⁺ RNAs. These were prepared from livers of rats that had been adrenalectomised 14 days previously, then received Dexamethasone by intraperitoneal injection 2 hours prior to sacrifice. Comparison with the relative abundances of ZE10 RNA in similar RNA preparations from livers of adrenalectomised rats that had not received

Figure 5: Effect of Dexamethasone on the relative abundance of ZE10 RNA. Poly(A)⁺ nuclear and polysomal RNAs from the livers of normal rats, rats adrenalectomised 14 days previously, and adrenalectomised rats which were sacrificed 2 h after receiving an injection of Dexamethasone (2 hr Dex) were dotted in a doubling dilution fashion with 0.2 µg of each RNA on the first position, and hybridised with ³²P-labelled ZE10 DNA. (Abbreviations as for Table 2).

Dexamethasone and from normal rats' livers showed that Dexamethasone rapidly reverses the effect of adrenalectomy. Within 2 hours of administration of the synthetic glucocorticoid there has been a 4 to 8-fold increase in the relative abundance of ZE10 RNA in polysomal poly(A)⁺ RNA and a concomitant 4 to 8-fold decrease in the relative abundance of the sequence in nuclear poly(A)⁺ RNA, to levels close to those found in normal rat liver polysomal and nuclear RNAs (Fig. 5).

DISCUSSION

The hybridisation analyses of Bajwa et al. (8) suggested that 7,000-9,000 sequences that are relatively rare on normal rat liver polysomes were not detectable 14 days after adrenalectomy; these analyses also suggested that some of the more abundant sequences were increased in abundance. A cDNA library representing poly(A)⁺ RNA from the liver polysomes of normal rats was constructed. It was differentially screened with ³²P-labelled cDNAs transcribed from rat liver polysomal poly(A)⁺ RNA from both normal and adrenalectomised rats, and we detected many RNA sequences which were depleted in abundance 14 days after adrenalectomy. However,

despite construction and screening of a cDNA library which represented total poly(A)⁺ RNA from the livers of adrenalectomised rats (data not shown), no sequences that increased in abundance following adrenalectomy were detected, though again a number of sequences that were depleted in abundance were found. This discrepancy is most probably due to the difficulties inherent in interpretation of molecular hybridisation analyses (8) that involve populations of RNAs.

Our results indicate that the steady-state levels of four RNAs complementary to the recombinants we selected are regulated by glucocorticoids to varying degrees and, moreover, that this regulation is, at least partially, at a post-transcriptional level. Fourteen days after adrenalectomy the relative polysomal RNA levels of 3 of them (ZE10, YE5 and XE5) are depleted, and the relative nuclear levels of these 3 are concomitantly increased.

Four possible explanations for these alterations in the relative abundances of these sequences were considered. Firstly, since we were studying the polyadenylated fractions of RNA in every case, there could have been an alteration in the proportion of each RNA sequence polyadenylated following adrenalectomy. This proved not to be so, since when we analysed the levels of the selected recombinants' homologous RNAs in the unpolyadenylated fraction of RNA from the nucleus and the polysomes, we found them to be unchanged two weeks after adrenalectomy.

Another possible explanation for an adrenalectomy-induced accumulation of RNA sequences in the nucleus is that a reduced level of glucocorticoids may lead to an inhibition of RNA processing. If this were so, Northern blot analysis would be expected to show an accumulation of higher molecular weight precursor RNAs in the liver nuclei from adrenalectomised rats. We have demonstrated, however, that it is the band corresponding to the mature $\alpha_2\mu$ -globulin size of 1.1 kb which increased in intensity in the nuclear RNA from the livers of adrenalectomised rats.

A third hypothesis is that the nuclear stability of our selected RNAs has been increased as a result of adrenalectomy. Steroid hormones have been shown to affect the stability of various RNAs in the cytoplasm (5,6,30) and more recently it was suggested by Vannice *et al.* (31) that glucocorticoids stimulate the nuclear stability of α -acid glycoprotein mRNA. It is quite possible that effects on nuclear RNA stability could be an important level at which gene expression is regulated (32). However, an increase in the stability of the nuclear sequences would not explain the concomitant

depletion on the polysomes which we observe in rat liver 14 days after adrenalectomy. An explanation in terms of stability would require that an increased nuclear stability was accompanied by concomitant decreased cytoplasmic stability of these mRNAs, an unlikely combination.

The fourth hypothesis is the one that we favour: depletion of circulating glucocorticoids leads to an inhibition of nucleo-cytoplasmic transport of some mRNAs. This mechanism would lead to accumulation of these mRNA sequences in the nucleus while the abundance of the RNAs on polysomes would be decreased due to turnover. Moreover, we have shown that, within two hours of an intraperitoneal injection of 2 mg of Dexamethasone, the abundance of ZE10 RNA in the nucleus is reduced while that on the polysomes is increased, to levels close to those in normal liver. The rapidity of this response supports our hypothesis that removal of glucocorticoids leads to an inhibition of the nucleo-cytoplasmic transport of ZE10 RNA, and injection of synthetic glucocorticoid stimulates the release of pre-formed ZE10 mRNA from the nucleus.

The insert in recombinant ZE10 was identified as encoding part of the 3' end of $\alpha_2\text{U}$ -globulin mRNA, the transcript from a member of a very closely related family of 18-20 differentially expressed genes (33). (From sequencing data the transcripts of these genes were found to show 97% homology (34) and were indistinguishable by molecular hybridisation). Recently, Feigelson and coworkers have shown that, in the rat liver, glucocorticoids regulate the transcription of $\alpha_2\text{U}$ -globulin genes (Feigelson, personal communication). However, their data do not exclude the possibility that the polysomal abundance of $\alpha_2\text{U}$ -globulin mRNA is modulated additionally by a post-transcriptional mechanism. Likewise, our data that show the polysomal abundance of this mRNA to be regulated post-transcriptionally do not rule out the possibility that glucocorticoids also regulate the transcription of the gene. Consequently, taken together, these data suggest that the expression of $\alpha_2\text{U}$ -globulin genes is subject to two-stage control by glucocorticoids, that is, the steady-state abundance of mRNA is dependent initially on transcription and then modulated by a nucleo-cytoplasmic transport regulatory step.

Hormone-dependent post-transcriptional modulation of mRNA abundance is not unprecedented. For example, the stability of cytoplasmic casein mRNA and the rate of transcription of the gene are both specifically increased by insulin, hydrocortisone and prolactin in mammary gland cultures (30). More directly pertinent is the observation by Schumm and Webb (7) that release of

$\alpha_2\mu$ -globulin mRNA from isolated nuclei is regulated by physiological concentrations of insulin, though they do not show whether the effect on nuclei is sequence-specific. We are uncertain as to the extent that the effect of glucocorticoids on nucleo-cytoplasmic transport is specific for particular sequences, though our data do indicate that ZE10 RNA and three other sequences are affected to different extents by glucocorticoid withdrawal. Moreover, nucleo-cytoplasmic transport of albumin mRNA appears relatively unaffected by glucocorticoids, as expected since glucocorticoids are reported not to affect the abundance of albumin mRNA in the liver (28).

Notwithstanding our uncertainty regarding the generality of the effect we observe, our data constitute the first direct demonstration that, *in vivo*, glucocorticoids regulate nucleo-cytoplasmic transport of mRNA, and that the expression of some genes is regulated, in part at least, by a hormone at a level other than transcription or mRNA stability. These data add to the accumulating evidence that sequence-specific selection of mRNAs at the level of nucleo-cytoplasmic transport does occur, and that this may be an important mechanism whereby the relative abundances of polysomal mRNAs is modulated (10).

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