Glucocorticoid-dependent Expression of the Albumin Gene in Adult Rat Hepatocytes*

Katsuhiko Nawa, Toshikazu Nakamura, Atsushi Kumatori, Chiseko Noda, and Akira Ichihara

From the Institute for Enzyme Research, School of Medicine, University of Tokushima, Tokushima 770, Japan

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In primary cultures of adult rat hepatocytes, transcription of the albumin gene, measured as incorporation of $[^{32}P]UTP$ into mRNA in isolated nuclei, decreased dramatically during culture without addition of serum and hormone, becoming almost negligible 10 h after plating. Of the hormones tested, dexamethasone (0.1 $\mu M$) prevented this decrease and restored the transcription within 2 h to the same level as that before culture. The half-maximum dose of dexamethasone for induction of transcription of the albumin gene was about 30 nM. The in vitro finding that expression of the albumin gene is strictly regulated by glucocorticoid was confirmed by an in vivo experiment in adrenalec-tomized rats showing that the transcription decreased markedly 14 days after adrenalectomy, but was restored rapidly by administration of hydrocortisone. This finding was also supported by identification of a glucocorticoid regulatory sequence from -50 to -62 base pairs between the TATA box and CAT box upstream of the 5'-end of the albumin gene. Cycloheximide inhibited the induction of transcription of the albumin gene by dexamethasone, suggesting that a rapidly induced mediator protein, which is also regulated by glucocorticoid, is involved in the induction of albumin gene expression by glucocorticoid. The albumin gene was also regulated by various other hormones besides glucocorticoid. Glucagon markedly enhanced the transcription induced by dexamethasone, although glucagon alone had no effect. Conversely, epinephrine suppressed stimulation of expression of the albumin gene by dexamethasone. Insulin and triiodothyronine had no effect on transcription of the albumin gene. From these findings we conclude that expression of the albumin gene depends strictly on glucocorticoid, and this dependence is modulated by other hormones.

Serum albumin is a major plasma protein, and its production is a typical differentiated function of hepatocytes. Albumin synthesis amounts to at least 10% of the total protein synthesis in rat liver and over 30% of the synthesis of serum proteins secreted by mature hepatocytes (1). Therefore, its mRNA constitutes as much as 10% of the total poly(A) RNA in adult rat liver (2). Because of the large pool size and high stability of albumin mRNA (3), nutritional and hormonal factors have been thought not to have marked effects on expression of the albumin gene in hepatocytes; and in fact, starvation (4) and administration of glucocorticoid (5) were reported not to cause marked change in the level of albumin mRNA in rat liver.

However, results in vivo are influenced by so many secondary factors that it is difficult to draw clear conclusions on mechanisms of hormone actions. To avoid the inevitable complexity of systems in vivo, we have been using primary cultures of adult rat hepatocytes in studies on hormonal regulation of gene expression of liver-specific enzymes such as tryptophan 2,3-dioxygenase (6-8) and serine dehydratase (9, 10). During these studies, we unexpectedly found that transcription of the albumin gene in hepatocytes was markedly stimulated by glucocorticoid; transcription of this gene decreased sharply when rat hepatocytes were cultured without any hormone and was restored to the level in vivo within 2 h when glucocorticoid was added to cultures. These results were confirmed in vivo by administration of hydrocortisone to adrenalec-tomized rats.

This is the first report that expression of the albumin gene is strictly regulated by glucocorticoid in rat hepatocytes. The dependence of gene expression on glucocorticoid could be understood by the finding that a glucocorticoid regulatory sequence, which was recognized by the glucocorticoid receptor complex, was present in the 5'-flanking region of the albumin gene. This paper also reports that stimulation of transcription of the albumin gene by glucocorticoid was enhanced by glucagon and suppressed by epinephrine.

**EXPERIMENTAL PROCEDURES**

**Materials**—The materials used for cell isolation and culture were as reported previously (11). Insulin and glucagon were obtained from Sigma. $R$-(-)-Epinephrine bitartrate was from Nakarai Chemicals, Kyoto. Dexamethasone was from Wako Pure Chemicals, Osaka. Hydrocortisone acetate was obtained from Schering AG, Berlin. Nitrocellulose filters (BA85) were purchased from Schleicher & Schüll, GmbH, Dassel. X-ray film (XAR-5) was from Eastman. $[^{32}P]CTP$ (3000 Ci/mmol) for nick translation and $[^{32}P]UTP$ (800 Ci/mmol) and $[^{3}]H]UTP$ (53 Ci/mmol) for transcription assay were from New England Nuclear. Plasmid pBR322 containing rat albumin cDNA (pcAlb; 1 1.2-kilobase pair insert) was a gift from Dr. Yasuyuki Takagi, Tohoku Gakuen University, School of Medicine, Nagoya.

**Isolation and Primary Culture of Hepatocytes**—Parenchymal hepatocytes from adult male Wistar rats, weighing 180-250 g and given laboratory chow ad libitum, were isolated by in situ perfusion of the liver with collagenase (11). The cells were suspended in Williams' medium E containing 5% calf serum and 2 mM insulin and were cultured at a density of 10^6 cells/cm^2 in Corning plastic dishes at 37 °C under 5% CO_2 in air. After 4 h, the medium was replaced by hormone- and serum-free medium. Test hormones were added to 20-h cultures in fresh medium.

**Assays of Secretion of Albumin and Content of Protein**—The secretion of albumin was measured with a enzyme immunoassay kit (Ohtsuka Assay Laboratory, Tokushima) using a specific antibody to

1 The abbreviations used are: pcAlb, pBR322 containing rat albumin cDNA; SDS, sodium dodecyl sulfate; MMTV, murine mammary tumor virus; SSPE, 0.18 M NaCl, 10 mM Na_2HPO_4 (pH 7.4), and 1 mM EDTA (pH 7.4).
rat serum albumin. Protein was measured by the method of Lowry et al. (12).

Preparation and Dot-blot Hybridization of RNA—Total RNAs of cultured hepatocytes and liver tissue were isolated by phenol/SDS extraction as described previously (13–15) with some modification (16). Total RNA was obtained from 10^7 cells, and 3 mg of RNA was extracted from 1 g of tissue. RNA dot-blot hybridization was performed essentially as described previously (17, 18). RNA was denatured with 1 M glyoxal, 10 mM sodium phosphate (pH 7.0) at 50 °C for 1 h, and the glyoxalated RNA was spotted onto nitrocellulose paper pretreated with 20 × SSC using a dot-blot template (Bio-Rad). The nitrocellulose paper was baked at 80 °C for 2 h and hybridized with nick-translated albumin cdNA insert. Before hybridization, the nitrocellulose paper was prehybridized in 50% formamide, 5 × SSPE (pH 7.4), 200 μg/ml sonicated salmon sperm DNA, and 5 × Denhardt's solution at 42 °C for 24 h. Hybridization was performed at 42 °C for 48 h in the same solution containing 10% dextran sulfate and nick-translated rat albumin cdNA (5 × 10^6 cpm/ml), which had a specific activity of 1.5–3.0 × 10^9 cpm/μg as described previously (19). After hybridization, the nitrocellulose paper was washed four times with 2 × SSC, 0.1% SDS for 10 min each at room temperature, then twice with 1 × SSC, 0.1% SDS for 30 min each at 65 °C, and finally with 0.2 × SSC, 0.1% SDS for 30 min at 65 °C. The nitrocellulose paper was dried and exposed to x-ray film at −80 °C using a Cronex intensifying screen (Du Pont). After autoradiography, the radioactivity of each dot was detected in a liquid scintillation counter.

Isolation of Nuclei—Nuclei were isolated from rat hepatocytes essentially as described previously (20, 21). Hepatocytes (4 × 10^7 cells) were washed with ice-cold phosphate-buffered saline (pH 7.4), harvested in Buffer I (0.32 M sucrose containing 10 mM Tris-HCl (pH 8.0), 5 mM CaCl_2, 2 mM magnesium acetate, 0.1 mM EDTA, 0.1% Nonidet P-40, 1.0 mM dithiothreitol, and 0.1 mM phenylmethylsulfon-yl fluoride) and stored in liquid nitrogen. The recovery by this procedure was approximately 90% of lo7 nuclei/100 μl in Buffer III (25% glycerol, 48 mM sodium phosphate, 4 mM magnesium acetate, 0.1 mM EDTA, 0.5 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride) and stored in liquid nitrogen. The recovery by this procedure was approximately 2 × 10^7 nuclei/4 × 10^7 cells.

Nuclei were isolated from rat liver essentially as described above. Rat liver was perfused with ice-cold 10 mM Tris-HCl (pH 8.0) containing 0.14 M NaCl, cut into small pieces, and homogenized in 10 volumes (v/w) of Buffer I in a Potter-Elvehjem-type homogenizer with a loose-fitting pestle. The homogenate was pooled, homogenized in 1 ml of Buffer I in a Potter-Elvehjem homogenizer, and filtered through 270 mesh nylon. The homogenate (1 ml) was mixed with 2 ml of Buffer II (2 mM sucrose containing 10 mM Tris-HCl (pH 8.0), 5 mM magnesium acetate, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride). The cell pellet was suspended in 2.0 ml of Buffer II and centrifuged at 50,000 × g for 60 min at 2 °C. The precipitated nuclei were suspended at 1–2 × 10^10 nuclei/100 μl in Buffer III (25% glycerol containing 50 mM Tris-HCl (pH 8.0), 5 mM magnesium acetate, 0.1 mM EDTA, 5.0 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride) and stored in liquid nitrogen. The recovery by this procedure was approximately 2 × 10^7 nuclei/4 × 10^7 cells.

Nuclei were isolated from rat liver essentially as described above. Rat liver was perfused with ice-cold 10 mM Tris-HCl (pH 8.0) containing 0.14 M NaCl, cut into small pieces, and homogenized in 10 volumes (v/w) of Buffer I in a Potter-Elvehjem-type homogenizer with a loose-fitting pestle. The homogenate was filtered through 150 mesh nylon to remove connective tissue and centrifuged at 500 × g for 5 min at 2 °C. The pellet containing nuclei was suspended in 2.0 ml of Buffer I and diluted with 5.0 ml of Buffer II. The mixture was layered onto 3.5 ml of Buffer II and centrifuged at 50,000 × g for 60 min at 2 °C. The precipitated nuclei were suspended at 1–2 × 10^10 nuclei/100 μl in Buffer III and stored in liquid nitrogen. The recovery by this procedure was approximately 5 × 10^7 nuclei/10 g of liver.

Transcription in Isolated Nuclei and Hybridization—Transcription in isolated nuclei, isolation of 32P-labeled RNA, and hybridization to nitrocellulose paper were performed as described previously (20, 21) with some modifications. For RNA synthesis, a suspension of nuclei (1–2 × 10^10 nuclei/100 μl of Buffer III) was incubated with 200 μl of reaction mixture containing 0.5 mM concentrations of NTPs (ATP, CTP, GTP, and TTP), 0.12 M KCl, 2.5 mM magnesium acetate, and 100 μCi of [α-32P]UTP at 25 °C for 40 min. The reaction was terminated by incubation with 15 μg/ml DNase I (RNase-free) at 25 °C for 20 min. The nuclei were solubilized by adding volumes of 1% SDS and 10 mM EDTA (pH 7.0), and then radiolabeled RNA was extracted with phenol/chloroform (2:1, v/v) at 65 °C (22) and precipitated with ethanol. The precipitated RNA was dissolved in 1 μl of 100 mM Tris-HCl (pH 7.6) containing 0.1 M NaCl, 0.1% SDS, and 1 mM EDTA. Unincorporated nucleotides were removed by chromatography on a Sephadex G-50 column. [32P]RNA transcripts were recovered with ethanol precipitation and dissolved in a small volume of H_2O. The radiolabeled RNA synthesized was hybridized to linearized pCAI (5 μg) on nitrocellulose paper. Prehybridization was performed at 42 °C for 12 h in 50% formamide, 5 × SSPE (pH 7.4), 5 × Denhardt's solution, 0.1% SDS, 100 μg/ml heat-denatured salmon

spem DNA, and 100 ng/ml poly(A), and then hybridization was performed in the same buffer (total volume, 500 μl) containing [32P]RNA transcripts at 42 °C for 72 h. [3H]cRNA prepared from pCAI as described by McKnight and Palmiter (23) was included in each hybridization mixture (2000 cpm/assay) to measure the hybridization efficiency. The nitrocellulose paper was washed in the same way as for RNA dot-blot hybridization as described above, and the [32P]RNA transcripts hybridized to each probe were counted in a liquid scintillation counter. The relative rate of transcription of the albumin gene was calculated as follows: cpm hybridized to (pCAI – pBR322)/cpm in total input RNA × 2100/1200 × 100/ hybridization efficiency × 10^5 (ppm). Mature rat albumin mRNA is 2100 nucleotides long (24), and pCAI used as a probe is 1200 base pairs long.

RESULTS

Effect of Glucocorticoid on Transcription of the Albumin Gene in Primary Cultures of Adult Rat Hepatocytes—When isolated rat hepatocytes were cultured in serum- and hormone-free medium, their albumin mRNA content slowly decreased in parallel with the rate of secretion of albumin during primary culture, as shown in Fig. 1. The rate of transcription of the albumin gene, however, rapidly decreased during culture and became almost negligible 10 h after plating. Clayton and Darnell (25) reported a similar sharp decrease in the transcription of many liver-specific genes including albumin in primary cultures of mouse hepatocytes. This decrease in the transcriptional rate was not due to damage in isolation of the hepatocytes because the synthesis of total RNA and protein by the hepatocytes did not decrease during isolation and culture of the cells (data not shown). We thought that this rapid decrease in transcription might be due to culture without hormones. Therefore, we examined whether any hormones could prevent the sharp decrease and stimulate transcription of the albumin gene. Results showed that dexamethasone prevented the decrease and greatly stimulated transcription of the albumin gene. Fig. 2 shows that addition of 0.1 mM dexamethasone to the fresh medium after a 20-h culture without hormones resulted in marked increase in the

FIG. 1. Changes in transcription of the albumin gene, the amount of albumin mRNA, and the secretion of albumin in adult rat hepatocytes during primary culture. Isolated hepatocytes were cultured in hormone- and serum-free medium. For transcription assay (nuclear run-on assay), nuclei were isolated at the indicated times and the transcription rate of the albumin gene was determined as described under "Experimental Procedures." The average hybridization efficiency was 22%. The relative amount of albumin mRNA (C) was determined by dot-blot hybridization as described under "Experimental Procedures." The amount of albumin mRNA was calculated as described in the legend of Fig. 1. The secretion of albumin in culture without hormones resulted in marked increase in the
rate of transcription of the albumin gene. The transcriptional rate increased rapidly to a maximum 2 h after the addition of dexamethasone and then decreased gradually. Fig. 3 shows the dose dependence of the effect of treatment of hepatocytes with dexamethasone for 2 h on induction of transcription of the albumin gene. The transcription increased dose dependently, and maximum stimulation was achieved with 0.5 μM dexamethasone. Half-maximum stimulation was observed with 30 nM dexamethasone.

To confirm the in vitro finding that gene expression of albumin in hepatocytes is strictly controlled by glucocorticoid, we examined the effect of glucocorticoid on transcription of the albumin gene in vivo. Fig. 4 shows that the rate of transcription of the albumin gene decreased to about 15% of the normal level 14 days after adenectomy. On the contrary, the amount of albumin mRNA decreased only slightly, remaining at 70% of the control level, indicating that albumin mRNA is relatively stable and that its pool size in hepatocytes is very large. This may be why nutritional and hormonal factors had no marked effect on the synthesis and secretion of albumin by rat liver. Administration of hydrocortisone 14 days after adenectomy caused rapid stimulation of transcription of the albumin gene; the transcription increased 3-fold 1 h after hydrocortisone administration and was restored to the normal level after 3 h. However, the amount of albumin mRNA was not restored as much as the transcription rate owing to its large pool size.

Identification of a Glucocorticoid Consensus Sequence in the 5'-Flanking Region of the Rat Albumin Gene—The above findings strongly suggest that the albumin gene should have a regulatory sequence recognized by glucocorticoid in its 5'-flanking region like that identified in murine mammary tumor virus (MMTV) (26), human metallothionein (27), and human growth hormone (28, 29) genes. Therefore, we examined the 5'-terminal sequence of the rat albumin gene determined by Sargent et al. (24). One possible sequence for the glucocorticoid receptor binding was found from base pairs −50 to −62 between the TATA and CAT boxes upstream of the 5'-end of rat albumin gene, as shown in Fig. 5. The sequence of the human albumin gene determined recently by Urano et al. (30) also contains a similar sequence in this region. The rat and human sequences differ slightly from the consensus sequences identified before, but both are likely to be involved in activation of the albumin gene by glucocorticoid.

Effects of Various Hormones and Cycloheximide on Transcription of the Albumin Gene—Next we examined whether other hormones affected transcription of the albumin gene in primary cultured rat hepatocytes. Table I shows that glucagon enhanced the stimulation of transcription by dexamethasone,

![Fig. 2](left). Time course of induction of transcription of the albumin gene by dexamethasone in primary cultures of rat hepatocytes. Hepatocytes were cultured in hormone- and serum-free medium, and 0.1 μM dexamethasone was added 20 h after plating. Nuclei were isolated at the indicated times, and transcription was assayed as described under “Experimental Procedures.” The average hybridization efficiency was 22%. Values are means of two experiments.

![Fig. 3](center). Dose dependence of effect of dexamethasone on transcription of the albumin gene in primary cultures of rat hepatocytes. One-day cultures of hepatocytes were incubated with the indicated concentrations of dexamethasone for 2 h. After isolation of nuclei, transcription assay was performed as described under “Experimental Procedures.” The average hybridization efficiency was 22%. Values are means of two experiments.

![Fig. 4](right). Effects of adenectomy and administration of hydrocortisone on transcription of the albumin gene and the amount of albumin mRNA in rat liver. Hydrocortisone acetate (5 mg/100 g of body weight) was injected intraperitoneally into rats 14 days after adenectomy. The rats were killed at the indicated times, and total RNA and nuclei were isolated from the livers. Transcription assay (open bars) and RNA dot-blot hybridization (solid bars) were performed as described under “Experimental Procedures.” The average hybridization efficiency was 22%. Values are means ± S.D. for three animals.

![Fig. 5](center). Sequence homology in the 5'-flanking region of the rat albumin gene with glucocorticoid regulatory sequences in other genes regulated by glucocorticoid. Sequences were aligned with the binding sites of glucocorticoid—its receptor complex in murine mammary tumor virus I and II (26), human metallothionein (h-MT) II (27), and human growth hormone (h-GH) (28, 29) genes. On the basis of these consensus sequences, possible glucocorticoid regulatory domains in the 5'-flanking regions of the rat (24) and human (30) albumin genes are also shown. The residues of the rat gene exhibiting homology with one or more of the consensus sequences are underlined.
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### TABLE I
Effects of various hormones on transcription of the albumin gene in primary cultures of adult rat hepatocytes

One-day cultures of hepatocytes were treated with the indicated hormones for 2 h, and then their nuclei were isolated. Transcription was assayed as described under “Experimental Procedures.” The average efficiency of hybridization was 22%. Values are means of two separate experiments.

| Hormones                        | Input of [32P]RNA (cpm x 10^4) | [32P]RNA hybridized (pAb - pBR322) | Relative rate of transcription (cpm -fold) |
|---------------------------------|---------------------------------|-----------------------------------|------------------------------------------|
| None                            | 14.9                            | 29                                | 15.6 (1.0)                               |
| Dexamethasone, 10^{-7} M        | 15.0                            | 203                               | 108.8 (7.5)                              |
| Insulin, 10^{-7} M              | 15.1                            | 25                                | 13.4 (0.9)                               |
| Glucagon, 10^{-4} M             | 16.5                            | 39                                | 15.6 (1.2)                               |
| Triiodothyronine, 10^{-4} M     | 13.2                            | 25                                | 15.1 (1.0)                               |
| Dexamethasone + insulin         | 14.7                            | 147                               | 79.8 (5.1)                               |
| Dexamethasone + glucagon        | 15.8                            | 333                               | 168.8 (10.8)                             |
| Dexamethasone + glucagon + insulin | 16.5                         | 192                               | 92.8 (5.9)                               |
| Dexamethasone + epinephrine, 10^{-4} M | 11.3                       | 82                                | 57.6 (3.7)                               |
| Dexamethasone + cycloheximide, 10^{-3} M | 14.7                      | 43                                | 28.4 (1.5)                               |

whereas epinephrine suppressed this stimulation by dexamethasone. Glucagon alone had no effect on transcription of the albumin gene. Insulin has been suspected to stimulate transcription of the albumin gene because it has been found to stimulate synthesis of albumin by increasing its mRNA level in vivo (31, 32) and in vitro (33). However, unexpectedly, insulin tended to inhibit transcription of the albumin gene, as shown in Table I. Thus, insulin may act on a post-transcriptional step. In fact, Fig. 6 shows that insulin significantly increased the amount of albumin mRNA in adult rat hepatocytes cultured under serum- and hormone-free conditions without stimulating transcription of the albumin gene. These results suggest that insulin acts by stabilizing albumin mRNA. Triiodothyronine had no effect on transcription of the albumin gene.

The fact that increase in transcription of the albumin gene by dexamethasone occurs more slowly than increase in transcription of MMTV, which occurs within 15 min of treatment with glucocorticoid (34), suggests that synthesis of a new protein is required for the action of glucocorticoid on expression of the albumin gene. To test this possibility, cultured rat hepatocytes were treated with dexamethasone for 2 h in the presence of cycloheximide (10 μM), which inhibits more than 95% of the protein synthesis of the cells. Cycloheximide markedly blocked the 7-fold increase in albumin transcription induced by dexamethasone, as shown in Table 1, but did not block total RNA synthesis (data not shown). These results suggest that the effect of dexamethasone on transcription of the albumin gene is mediated through synthesis of a new protein.

**DISCUSSION**

The hormonal regulation of serum albumin synthesis in liver has been studied for many years (35–37). However, it is difficult to obtain clear-cut results on hormonal effects on albumin synthesis in rat liver because of the complexity of conditions in vivo as well as the large pool size and stability of albumin mRNA in the liver. In this work, we showed that transcription of the albumin gene in rat hepatocytes was markedly stimulated by dexamethasone and that the effect of dexamethasone was enhanced by glucagon and suppressed by epinephrine. We confirmed the glucocorticoid-dependent expression of the albumin gene by identifying a glucocorticoid consensus sequence in the 5'-flanking region of the albumin gene. The main reasons why we could demonstrate that expression of the albumin gene is strictly regulated by glucocorticoid and is also modulated by other hormones are thought to be as follows. 1) We did not focus to the amount of albumin mRNA, which is present in large amounts in liver, but on the transcription of the albumin gene. 2) We used hormone- and serum-free primary cultures of adult rat hepatocytes to avoid the influence of endogenous hormonal effects and the complexity of conditions in vivo.

Previous studies in vitro using a mouse hepatoma cell line showed that glucocorticoid stimulated albumin synthesis by increasing its mRNA (38, 39). However, there have been no studies on whether glucocorticoid has a direct effect on expression of the albumin gene at the transcriptional level. Moreover, albumin synthesis is much lower in hepatoma cells than in normal adult hepatocytes. The hormonal responses, if any, in the hepatoma cell line, may be different from those of cells in vivo; and since these cells are cancer cells, they are by no means normal. In contrast, adult rat hepatocytes in primary culture show the normal functions of liver cells in vivo (40–42), and their ability to synthesize albumin is almost the same as that of rat liver in vivo (43).

Much progress in studies on the action of glucocorticoid on gene expression has been achieved by studies on the transcription of MMTV (28, 44, 45). Glucocorticoid increases the transcription of MMTV up to 100-fold within 15 min (34);

![Fig. 6. Effect of insulin on the amount of albumin mRNA in adult rat hepatocytes during primary culture. Hepatocytes were cultured with or without 0.1 μM insulin. On the indicated days, the cells were harvested and their total RNA was extracted. RNA dot hybridization assay was performed as described under “Experimental Procedures,” and the radioactivity of nick-translated albumin cDNA hybridized to RNA on nitrocellulose paper was determined. Data are expressed as percentages of control values on the indicated days.](image-url)
and its effect is independent of protein synthesis, indicating that it has a direct effect on the MMTV gene. In contrast, we found that cycloheximide blocked the stimulatory effect of dexamethasone on transcription of the albumin gene (Table 1), suggesting that a rapidly induced protein may be required for the action of glucocorticoid on the albumin gene. This possibility was supported by the findings that induction of the maximum rate of transcription of the albumin gene took much longer than that of the MMTV gene after hormone addition (Fig. 2) and that induction of transcription of the albumin gene by administration of hydrocortisone was much slower in adrenalectomized rats (Fig. 4) than in normal rats, in which it occurred within 15–30 min. Similarly, cycloheximide is known to block activation of the genes for α2, -globulin (46), α1-acid glycoprotein (47, 48), and phosphoenolpyruvate carboxykinase (49), which are regulated by glucocorticoid. Recently, we found that stimulation of the transcription of tryptophan 2,3-dioxygenase and serine dehydratase genes by dexamethasone in primary cultured hepatocytes was also blocked by cycloheximide. We also found that the time when the stimulation of these gene expressions by dexamethasone reached a maximum was much later in primary cultures that did not contain hormones before addition of dexamethasone. These findings suggest that a short-lived mediator protein, which is also regulated by glucocorticoid, is required in the mechanism of action of glucocorticoid inactivating many liver-specific genes such as those of albumin, tryptophan 2,3-dioxygenase, and serine dehydratase.

The present work showed that expression of the albumin gene was modulated by various other hormones besides glucocorticoid. As in the cases of other liver-specific enzymes such as tryptophan 2,3-dioxygenase and serine dehydratase, glucagon alone did not increase transcription of the albumin gene, but enhanced the effects of dexamethasone on transcription. Recently, many studies have shown that cAMP is involved in stimulation of transcription (50–52). A cAMP regulatory region was found at the 5′-end of the phosphoenolpyruvate carboxykinase gene, which is similar to the consensus sequences found in all prokaryotic genes regulated by cAMP (53). However, details of the molecular mechanism of the action of cAMP on gene expression are unknown.

Epinephrine suppressed the stimulatory effect of dexamethasone on the transcription of the albumin gene. This suppressive effect of epinephrine may be mediated by the α1-adrenergic receptor like its suppressive effects on the induction of tryptophan 2,3-dioxygenase (7) and serine dehydratase (9, 10). Epinephrine has been shown to stimulate turnover of phosphatidylinositol (54) and to increase the intracellular Ca2+ concentration through the α1-adrenergic receptor (55), but it is still unknown whether this mechanism is involved in its suppressive effect on expression of the albumin gene.

There has been much recent progress in studies on regulation of gene expression in eukaryotic cells. In studies on hormonal regulation of gene expression, we are now confronted with two problems. One is the intracellular signal transduction from the hormone receptor to nuclear chromatin; the other is the identification of trans-acting transcriptional factor regulated by hormone. Introduction of a cloned gene into hormone-sensitive cells and development of an in vitro transcription system regulated by the hormonal signal will allow us to solve these problems.

K. Nawa, T. Nakamura, A. Kumatori, C. Noda, and A. Ichihara, unpublished data.
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