Xenopus Fibrinogen

CHARACTERIZATION OF SUBUNITS AND HORMONAL REGULATION OF BIOSYNTHESIS*

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In this paper we describe the purification and characterization of Xenopus plasma fibrinogen and the hormonal factors which regulate synthesis and secretion of fibrinogen in liver parenchymal cells in primary culture. As in other vertebrate species, Xenopus fibrinogen is composed of three nonidentical polypeptide chains, Aa, Bβ, and γ. In contrast to mammalian fibrinogens, the Bβ chain of Xenopus fibrinogen has a higher apparent molecular weight than the Aa chain. The γ chain has the lowest molecular weight in the frog protein, as in that of other species. The relatively large size of the frog Bβ chain results from the unusually large size of the NH₂-terminal B fibrinopeptide, which is released by thrombin cleavage of fibrinogen.

Hormonal regulation of fibrinogen biosynthesis was examined using a primary cell culture system. Purified Xenopus liver parenchymal cells, maintained for several weeks in a defined culture medium, gradually decrease the synthesis and secretion of fibrinogen. Sustained production of this protein is dependent upon the addition of a glucocorticoid, dexamethasone, to the culture medium. Fibrinogen production is suppressed if an estrogen, estradiol-17β, is added to the culture medium together with dexamethasone and triiodothyronine. The Xenopus system provides new insight into the structure of fibrinogen, the evolution of this protein, and the hormonal factors which regulate its synthesis.

Fibrinogen is the soluble precursor of fibrin, which forms the insoluble matrix of a blood clot. Synthesis of fibrinogen occurs in the liver. It is one of the acute phase proteins whose levels in the plasma increase in response to injury or stress as a result of increased biosynthesis (1-6). Plasma levels of adrenal hormones, including glucocorticoids (7, 8), also increase during the acute phase response. Glucocorticoids act directly on the liver to regulate the synthesis and secretion of fibrinogen (9-13).

Primary cultures of Xenopus liver tissue and purified parenchymal cells can be maintained for several weeks in defined medium (14-16). Under these conditions, the cells cease the production of many major secreted proteins, but they remain responsive to hormonal stimulation. We have shown that estradiol-17β added to the culture medium induces the synthesis and secretion of vitellogenin, the precursor of the major egg yolk proteins (14). Thyroid hormones and glucocorticoids function as co-hormones to enhance estrogen induction of secreted vitellogenin (15, 16). When the glucocorticoid dexamethasone is added to the culture medium alone, it acts to sustain or induce the synthesis and secretion of several proteins which are normally secreted by freshly excised liver (14, 16).

In all vertebrate species which have been studied, fibrinogen is a multimeric molecule with a total molecular weight of about 340,000 (for reviews see Refs. 17 and 18). Each molecule is composed of two sets of three nonidentical subunits designated Aa, Bβ, and γ. In mammalian species, the relative sizes of the subunits, from largest to smallest, is Aa, Bβ, and γ. During the coagulation process, the proteolytic enzyme thrombin cleaves two small polypeptides called the A and B fibrinopeptides from the NH₂-termini of the Aa and Bβ fibrinogen chains, respectively. The resulting molecules of fibrin polymerize to form the matrix of the blood clot.

In this communication we describe the isolation and characterization of fibrinogen from Xenopus plasma. Our characterization of frog fibrinogen has allowed us to identify the fibrinogen polypeptides synthesized and secreted by liver cells in culture and to analyze the hormonal factors which regulate fibrinogen biosynthesis in this system.

EXPERIMENTAL PROCEDURES

Preparation of Xenopus Fibrinogen—Adult female frogs, 20 animals, were anesthetized by immersion in 0.2 g/100 ml of ethyl m-amino benzoate, methanesulfonic acid salt (Aldrich). The animals were dissected abdominally exposing the heart, taking care to avoid the central vein. Heparin (Sigma type H7005), 0.1 ml of a 1500-unit/ml solution, was injected into the heart and about 5 ml of blood was withdrawn into a syringe rinsed with the heparin solution. One-tenth volume of the following anticoagulant solution was added to the whole blood on ice: 2 m ε-amino caproic acid, 90 units/ml of heparin, 1 m NaCl, 0.2 m EDTA, 0.1 m sodium phosphate, pH 7.3, and 1/100th volume of freshly prepared phenylmethanolsulfonyl fluoride, at 15 mg/ml in absolute ethanol. Cells were removed by centrifugation for 10 min at 3500 rpm. The plasma was treated with BaSO₄, followed by triethylaminoethylcellulose to adsorb contaminating proteins (19). Addition of glycine to the soluble proteins failed to precipitate fibrinogen, as expected for mammalian preparations (20). Precipitation was

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accomplished by the procedure of Bergström and Wallén (21). The resulting pellet was redissolved as a concentrated solution. Fibrinogen was then further purified by precipitation with glycine (30). The final protein pellet was dissolved and dialyzed at 4 °C in order to remove the cryoprecipitate (22). The purified protein was stored at -20 °C in 0.3 M NaCl, 10 mM Tris-HCl, pH 7.3. The yield of purified fibrinogen was 35 mg/100 ml of plasma. Upon addition of thrombin, 95% of the purified protein formed a fibrin clot. Protein concentration was measured by the method of Lowry et al. (23). Bovine fibrinogen was purified as described (24). Human fibrinogen was a gift from Dr. M. Mosesson (Department of Medicine, Mount Sinai Medical Center, Milwaukee, WI).

Selective Enzymatic Digestion of Fibrinogen—For experiments in which unfractionated Xenopus plasma was digested with thrombin, the plasma was collected using 0.6 x 0.15 M NaCl, 0.015 M sodium citrate without other protease inhibitors. Bovine thrombin was purified according to the procedure of De Vreker et al. (25). The preparation started with 20,000 NIH units of thrombin and the final purified material was dissolved in 0.3 M NaCl, 0.02 M Na phosphate, pH 7.4, to give an A300 nm of 0.15. Batroxbolin (Pentapharm, Basel, Switzerland) at 500 BU/mg was prepared as a stock solution at 1 mg/ml in 0.15 M NaCl. Copperhead Venzyme was obtained from Dr. J. Shainoff (Thrombosis Research Section, The Cleveland Clinic Foundation, Cleveland, OH), as a stock solution at 15 a-N-tosyl-L-arginine methyl ester units/ml (26). Digestion reactions contained 7.5 µg of fibrinogen in 20-50 µl with final concentrations of 0.015 M Tris-HCl, pH 7.5, and 0.14 M NaCl. Digestion of radioactively labeled secreted fibrinogen was accomplished by including up to one-half volume of tissue culture medium in appropriate reactions. Amounts of enzymes used and incubation conditions are given in the figure legends. During the course of these investigations we observed that the ability of Venzyme to selectively digest only the Bβ polypeptide of Xenopus fibrinogen was enhanced by addition of the solution used in an in vitro translation system (27). For this reason the enzyme digestion with Venzyme also contained one-tenth volume of: 25 mM of each amino acid (except methionine), 1 mM ATP, 0.2 mM GTP, 8 mM creatine phosphate, 24 mM Hepes1, pH 7.6, 600 µM spermidine, 2 mM diithiothreitol, 120 mM potassium acetate, and 2 mM magnesium acetate. Preliminary experiments suggest that this enhanced specificity is due to the inhibition of a secondary protease activity present in the preparation of Copperhead Venzyme which digests the Aα fibrinogen polypeptide of Xenopus but does not digest the Aα polypeptide of either human or bovine fibrinogen.

Liver Cell Cultures—Liver parenchymal cells were prepared and purified as described previously (14) with the following modifications. Enzymatic perfusion utilized 25 ml of Type 1 collagenase (Sigma) at 200 units/ml without inclusion of bovine serum albumin and the disaggregation was completed in vitro with an additional 50 ml of the enzyme solution. No tissue pulp remained after this treatment. The final cell washes were carried out using 60% modified Coon’s Medium in each well was changed every other day. Steroid hormones were dissolved as 25x stocks and were added to cultures as indicated in the text. Radioactive labeling of secreted proteins with [35]methionine was carried out as described (15) except that heparin (1500 units/ml) was included in the labeling medium to prevent the degradation of fibrinogen as described by Grieninger et al. (13). For some experiments, cells were taken from a normal adult male animal while in other experiments cells were prepared from a male injected with 2 mg of estradiol-17β either two weeks or more than three months prior to sacrifice, as indicated in the figure legends.

Immunoprecipitation of Fibrinogen—A rabbit antiserum was prepared using the procedure of purified fibrinogen. The protein at 3.9 mg/ml in 0.3 M NaCl, 10 mM Tris-HCl, pH 7.3, was dialyzed with an equal volume of Freund’s complete adjuvant (Difco) and 780 µg of fibrinogen was injected intradermally. Five weeks later, the animal was injected with 400 µg of fibrinogen in Freund’s incomplete adjuvant and was bled seven days later. The resulting antiserum was used without further purification except that heparin and phenylmethylsulfonylfluoride, final concentrations 15 and 150 µg/ml, respectively, were added before use to inhibit rabbit thrombin activity. Immunoprecipitations were carried out using Staphylococcus aureus to precipitate the antigen-antibody complex according to the method of Kessler (28).

Polyacrylamide Gel Electrophoresis and Autoradiography—SDS-polyacrylamide gel electrophoresis and autoradiography were carried out as described previously (15). Gels were poured as exponential gradients using acrylamide (Eastman) stock solutions in the proportions of 2 volumes of 9 g/100 ml : 1 volume of 15 g/100 ml. Samples were dissolved in a modified sample buffer containing: 62.5 mM Tris-40 mM EDTA, pH 6.8, 2 g/100 ml SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 300 mM 2-mercaptoethanol sulfonic acid, 0.025 g/100 ml bromphenol blue. Protein molecular weights were determined by comparison with molecular weight standards purchased from Bethesda Research Laboratories.

RESULTS AND DISCUSSION

Fibrinogen Structure

Resolution of Xenopus Plasma Fibrinogen Subunits—Fibrinogen purified from Xenopus plasma is resolved by electrophoresis into four polypeptide bands (Fig. 1, lane C). The apparent molecular weights of these polypeptides are 63K, 59K, 55K, and 52K. The same protein bands are observed in whole plasma (Fig. 1, lane B), but the relative amount of the 59K polypeptide decreases and that of the 55K polypeptide increases during the process of fibrinogen purification. These observations suggest that the 59K polypeptide is converted to the 55K polypeptide and that this process continues during the protein isolation procedure. This hypothesis is supported

1 The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate. 63K, 59K, 55K, etc., M, = 63,000, 59,000, 55,000, etc.

FIG. 1. Identification of Xenopus fibrinogen polypeptides in total plasma and purified fibrinogen. Coomassie blue-stained proteins after SDS-polyacrylamide gel electrophoresis of: total plasma digested with thrombin (lane A); total plasma, undigested (lane B); purified fibrinogen, undigested (lane C); purified fibrinogen, digested with thrombin (lane D). Thrombin digestion was carried out using a 1:10 dilution of stock solution, at 37 °C for 1 h.
Identification of Plasma Fibrinogen Subunits—In order to relate the four observed polypeptides to the three subunits of fibrinogen, we investigated which polypeptide bands are sensitive to digestion with specific proteolytic enzymes known to selectively digest particular subunits of fibrinogen. Thrombin cleaves the A and B fibrinopeptides from the Aα and Bβ subunits of fibrinogen, giving rise to the α and β chains of fibrin. The γ subunit is not digested by this enzyme. Addition of thrombin-dependent polypeptide cleavages indicates that the three largest polypeptides are forms of the Aα and Bβ subunits, while the 52K polypeptide is the γ subunit.

Specific identification of the frog Aα and Bβ subunits was accomplished by the technique of selective enzymatic digestion of subunits using snake venom proteases. The enzymes batroxobin and Venzyme selectively digest the Aα and Bβ subunits of fibrinogen, respectively (26, 30, 31). Human, bovine, and frog fibrinogens were digested with these enzymes and the subunits of each fibrinogen were separated electrophoretically and examined for the disappearance of particular bands as well as the appearance of cleavage products. In our gel system, undigested human fibrinogen is resolved into five bands: a triplet of bands for the Aα subunit, one Bβ band, and one γ band (Fig. 2, lane —). Intact bovine fibrinogen is resolved into three bands, one Aα, one Bβ, and one γ band (Fig. 2, lane —), plus a minor band above γ which may be the γ form of this subunit (29). The subunits from all three species migrate in the same molecular weight range.

Digestion of both human and bovine fibrinogens with batroxobin, results in cleavage of the largest polypeptides only, and in the appearance of new bands of slightly lower molecular weight (Fig. 2, lane B). This control result confirms that, under our experimental conditions, batroxobin digestion is selective for the Aα subunit and that the slow-migrating polypeptides are the Aα chains in these two mammalian species. In contrast, digestion of Xenopus fibrinogen with batroxobin results in cleavage of the two middle bands, but not of the largest polypeptide. Thus, both of the middle two polypeptides are forms of the Aα subunit of Xenopus fibrinogen.

Batroxobin fails to digest the largest polypeptide of Xenopus fibrinogen. Since this chain is digested by thrombin, it must be the Bβ subunit. Selective digestion of fibrinogen with Venzyme was used to prove this deduction. Control digestions using human and bovine fibrinogens demonstrate that for each of these species only the middle band is digested (Fig. 2, lane V). Although Venzyme does not digest these polypeptides to completion under our conditions, it is specific for Bβ subunits. In contrast to mammalian fibrinogen, treatment of Xenopus fibrinogen with Venzyme results in digestion of the slowest migrating band. This finding directly confirms that the largest polypeptide of Xenopus fibrinogen is the Bβ subunit.

In summary, by the criterion of selective sensitivity to three complementary enzymes, we conclude that the 63K polypeptide is the Bβ subunit of Xenopus fibrinogen, while the 59K and 55K polypeptides are alternate forms of the Aα subunit. The 52K polypeptide, which is not cleaved by any of the proteolytic enzymes employed, is the γ subunit.

The Aα subunit of other species is known to be susceptible to proteolytic degradation at the COOH-terminal end (37), but the observed reversal in the relative sizes of the Xenopus Bβ and Aα subunits is not due to partial proteolytic degradation of Aα chains. This conclusion is based on the fact that the Aα chain of newly secreted fibrinogen molecules migrates with the larger form of the plasma Aα subunit, but is smaller than the secreted Bβ subunit (see below). Two forms of the Xenopus plasma Aα subunit are observed. Upon cleavage of the NH2-terminal fibrinopeptide with thrombin, both forms are decreased in molecular weight by the same amount. Thus, we conclude that the smaller Aα form arises from the primary secreted polypeptide by removal of a 4K dalton COOH-terminal peptide.

The Sizes of the B and A Fibrinopeptides—The large Bβ chain is accounted for by the unusually large size of the Xenopus B fibrinopeptide. Fibrinopeptides of mammals display some of the most highly variable protein sequences known (32), yet these peptides are relatively constant in size with nearly all of them comprising 13–21 amino acids and thus having molecular weights in the range of 1000–2000. Our results (Fig. 2) demonstrate that cleavage of the human Bβ chain yields a β polypeptide with a molecular weight approximately 1500 less. Similarly, in the case of bovine fibrinogen,
the β chain is about 2000 daltons smaller than the corresponding Bβ subunit. In contrast, thrombin cleavage of the Xenopus Bβ subunit gives rise to a β chain which is 6500 daltons smaller (63K to 56.5K). Thus, the apparent molecular weight of the frog B fibrinopeptide is several times greater than the size of mammalian B fibrinopeptides.

Although the Xenopus Bβ subunit differs from that of mammals, available evidence indicates that it is similar to that of other nonmammalian vertebrates. In the cases of chicken fibrinogen (39), and salmon fibrinogen (40), the subunits have been resolved and the Bβ has been shown to be larger than the Aα subunit. In another species of frog, Rana esculanta, the fibrinopeptide B has been reported to contain 43 amino acids, including tyrosine-O-SO₄ (41). This modified amino acid is characteristic of the B fibrinopeptides of many mammals and has not been found in any A fibrinopeptides. The total molecular weight calculated for these amino acids is 4387. Thus, an increased number of amino acids could partially account for the apparent size of the Xenopus B fibrinopeptide. Post-translational addition of carbohydrate residues at a site within the B fibrinopeptide accounts for the additional increase in molecular weight.  

In the lamprey, one of the most primitive extant vertebrates, both the size and modification of the B fibrinopeptide are similar to the structure we have observed for the frog. It comprises 36 amino acids, including tyrosine-O-SO₄, and it is glycosylated (42, 43). The structure of fibrinogen in the lamprey has proven difficult to analyze, but two alternate interpretations of the data suggest that the primary form of the Aα subunit is smaller than the Bβ subunit (44, 45).

The A fibrinopeptide of frogs is similar in size to that of higher vertebrate species and distinctly different from that of the lamprey. Each of the two forms of the Xenopus plasma Aα subunit gives rise to an α chain which is 2000 daltons smaller (59K to 57K and 55K to 53K) (Fig. 2). This result is consistent with the Rana A fibrinopeptide which contains 15–17 amino acids with a total molecular weight of about 1700 (41). The A fibrinopeptides of at least 40 mammals, and of the chicken, and a lizard, have been sequenced. In all cases they contain 13–19 amino acids (32, 38). In contrast, the lamprey A fibrinopeptide is only six amino acids long (43).

These comparisons of nonmammalian and mammalian fibrinogen polypeptides and fibrinopeptides suggest that major alterations have occurred in the length and modification of the fibrinopeptides during the course of evolution. Such changes are distinct from the frequent amino acid substitutions which characterize the fibrinopeptides of mammals (32). We hypothesize that the A fibrinopeptide may have increased in length while the B fibrinopeptide of nonmammalian vertebrates may have arisen as an extended, glycosylated peptide and this structure persisted until emergence of the mammals. If this view is correct, fibrinogen molecules with A and B fibrinopeptides of approximately equal length may be restricted to the mammals. Changes in the length and modification of fibrinopeptides can be expected to have dramatic effects on the solubility of fibrinogen molecules. These concepts can be tested by characterization of the fibrinogens from other nonmammalian vertebrate species.

**Fibrinogen Synthesis and Hormonal Regulation**

**Liver Cell Secreted Fibrinogen**—Characterization of fibrinogen from Xenopus plasma enabled us to identify fibrinogen secreted by liver cells in culture. We have previously described methods for the purification of Xenopus liver parenchymal cells and the establishment of long-term primary cultures (15). The cells in these cultures synthesize and secrete into the culture medium proteins which can be detected by radioactive labeling with [35S]methionine followed by gel electrophoresis and autoradiography. The pattern of radioactive secreted proteins includes three bands that co-migrated with the polypeptides of purified fibrinogen (Fig. 3, lanes A and D). In the region of the two plasma Aα chains there is only one radioactive band which co-migrates with the larger form of this subunit. These radioactive liver secreted proteins were confirmed to be subunits of fibrinogen by immunoprecipitation of secreted polypeptides with a rabbit antiserum prepared against plasma fibrinogen. Autoradiography of immune precipitates of radioactively labeled secreted proteins demonstrated that the anti-fibrinogen serum selectively precipitates the radioactive fibrinogen polypeptides (Fig. 3, lane B). These polypeptides are not precipitated by nonimmune serum (Fig. 3, lane C).

We used the method of selective enzymatic digestion with several proteases to identify the individual subunit polypeptides of secreted fibrinogen. The results of this analysis are shown in Fig. 4. As in the case of plasma fibrinogen, Venzyme digests the 63K radioactive band generating the 56.5K polypeptide (Fig. 4, lane V). Hence, these are the Bβ subunit and the β chain, respectively. Batroxobin cleaves the 59K band, identifying it as the Aα subunit, and generates the α chain at

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2 J. A. Gladner, personal communication.

3 L. J. Holland and L. J. Wangh, manuscript in preparation.
57K (Fig. 4, lane B). Thrombin digests both the 63K and the 59K secreted protein bands (Fig. 4, lane T). The 52K radioactive band is not cleaved by any of these enzymes and hence is the γ subunit. All of the cleavage products co-migrate with the subunit chains of plasma fibrin.

These results demonstrate that even in newly synthesized molecules the Bβ subunit of Xenopus fibrinogen is larger than the Aα subunit. Thus, the unusual relative sizes of the subunits does not result from proteolytic cleavage in the plasma. The secreted Aα subunit exists in a single 59K form. This fact supports our conclusion that the 55K plasma form is derived from the 59K polypeptide.

**Hormonal Regulation of Fibrinogen Synthesis—Xenopus**

provides an attractive experimental system in which to investigate the complex factors which regulate liver protein synthesis (14-16). In vivo experiments demonstrate that fibrinogen biosynthesis can be both positively and negatively regulated. The Xenopus liver primary cell culture system that we have developed has the experimental advantage that either purified parenchymal cells or pieces of tissue can be maintained for several weeks in a fully defined culture medium free of steroid and thyroid hormones. It is therefore possible to eliminate the action of endogenous hormones present in the cells at the start of the culture period.

**Glucocorticoid Stimulation of Fibrinogen Synthesis**—

When liver parenchymal cells are maintained in a defined medium lacking steroid hormones, they cease the synthesis of almost the entire set of normal secreted proteins. Synthesis and secretion of this set of proteins is sustained if dexamethasone is present. Addition of dexamethasone several weeks after the start of the culture period reinduces the synthesis and secretion of these proteins. The polypeptides of fibrinogen are among those secreted proteins whose synthesis is most rapidly responsive to the absence or presence of dexamethasone.

The results shown in Fig. 5 demonstrate these phenomena. Three sets of duplicate cultures were established in a defined medium lacking steroid hormones. Two days later one set of duplicates was given dexamethasone and the added hormone was maintained at each change of the culture medium. All cultures were maintained for 21 days, at which time another set of cultures received dexamethasone. Twenty-four hours later, secreted protein synthesis was monitored in all six cultures. The autoradiogram of the separated proteins secreted into the culture medium demonstrates that, in cultures which did not receive dexamethasone, the level of most secreted proteins had decreased significantly (Fig. 5, lanes −DX).

Cells cultured in the continuous presence of dexamethasone (Fig. 5, lanes +DX) continued to produce high levels of the entire set of secreted proteins. Cultures receiving the glucocorticoid 24 h before being assayed (Fig. 5, lanes −→ +DX) were reinduced to synthesize fibrinogen and some other secreted proteins. All three subunits of fibrinogen appear coordinately, consistent with the concept that these liver cells secrete intact fibrinogen molecules. Our findings complement and extend studies which have been carried out using liver from other animals (9, 11, 13).

The dose of dexamethasone used here was either 10⁻⁸ or 10⁻⁷ M. Even lower doses of dexamethasone, 10⁻⁹ M, are also effective if thyroid hormones are added as well (16). When liver cells are cultured for extended periods in the absence of added glucocorticoids, the addition of thyroid hormones alone has no effect on the pattern of secreted proteins. Thus, thyroid hormones appear to enhance the action of low levels of endogenous glucocorticoids or exogenously added dexamethasone, but thyroid hormones do not act alone as inducers of specific secreted proteins (15). These observations may explain reports indicating a direct effect of thyroid hormones on fibrinogen synthesis in relatively short-term primary cultures of chick liver cells (33).

Insulin has also been reported to induce increases in liver synthesis of fibrinogen and other secreted proteins in short-term primary cultures of chick liver cells (34). Insulin at 12.5 × 10⁻⁶ units/ml is present in all our culture media. Omission of insulin results in an overall decrease in the incorporation of the radioactive precursor into liver secreted proteins and in a generally less healthy appearance of these cells. Insulin alone does not induce or sustain the synthesis of specific secreted proteins.

**Estrogen Suppression of Fibrinogen Synthesis**—

Estrogens regulate frog liver production of vitellogenin, the precursor of major egg yolk proteins (35). Extensive hormone treatment induces very high levels of vitellogenin in the plasma of male animals, as well as the disappearance of virtually all other plasma proteins (36). These dramatic alterations in plasma protein composition reflect biosynthetic changes in the liver. Livers from males given chronic estrogen treatment synthesize and secrete vitellogenin, but not the normal set of secreted proteins.
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proteins (16). In an effort to understand the hormonal and cellular basis for this suppression, we have previously shown that cultured pieces of tissue from an estrogen suppressed animal are dependent on dexamethasone for reinduction of fibrinogen and other normal proteins (16). Reinduction failed to occur, however, in the presence of estradiol-17β, triiodothyronine, and dexamethasone, the combination of hormones which induces the highest level of vitellogenin production.

The results presented in Fig. 6 demonstrate that estrogen suppression can be achieved in cultures of isolated liver cells from a normal male animal. If cells are maintained for several weeks in the absence of steroid hormones, they show decreased secretion of most normal proteins, particularly fibrinogen (Fig. 6, lanes A). If a mixture of estradiol-17β, dexamethasone, and triiodothyronine is then added, within 24 h the estrogen induces the synthesis of vitellogenin while the glucocorticoid induces the synthesis of fibrinogen and several other proteins (Fig. 6, lanes B). The thyroid hormone enhances the actions of both steroids (15, 16). When the mixed hormone treatment is extended for one week, vitellogenin production is sustained at a high level while the synthesis and secretion of fibrinogen in particular is suppressed (Fig. 6, lanes C). These results demonstrate that estrogen suppression occurs by a mechanism operative within isolated normal liver cells, despite the fact that these cells are exposed to, and initially respond to, inducing levels of dexamethasone.

Suppression could arise by a failure of liver cells either to transcribe or to translate the messenger RNAs for particular proteins. We have examined the polypeptides translated in vitro from mRNA prepared from the livers of both estrogen-induced and glucocorticoid-induced or normal animals. Estrogen-induced RNA is depleted of translatable messages for serum albumin and the fibrinogen polypeptides. This observation, together with the fact that liver cells from estrogen-suppressed animals can be reinduced by dexamethasone to synthesize these proteins, lead us to hypothesize that both estrogen suppression and glucocorticoid induction occur by mechanisms involving gene transcription. Experiments are currently underway to test this hypothesis.

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