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Differential, Multihormonal Regulation of the Mouse Major Urinary Protein Gene Family in the Liver

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The hormonal requirements for the regulation of the major urinary protein (MUP) mRNA levels in mouse liver have been examined. Previous experiments have shown that administration of testosterone to female or castrated male mice increases MUP mRNA levels approximately fivefold to normal male levels. We have found that thyroxine and the peptide hormone, growth hormone, each had a pronounced effect on MUP mRNA levels. MUP mRNA was reduced 150-fold in growth-hormone-deficient mutant mice (*little*). The administration of growth hormone and thyroxine induced MUP mRNA approximately 150-fold, and when administered together, they induced MUP mRNA approximately 1,000-fold. testosterone administration. When administered separately to these mice, growth hormone and thyroxine induced with MUP mRNA approximately 150-fold, and when administered together, they induced MUP mRNA approximately 1,000-fold. Testicular feminized mice, which lack a functional major testosterone receptor protein, can also be induced to male levels by treatment with both growth hormone and thyroxine. In addition, we present evidence which indicates that growth hormone, thyroxine, and testosterone differentially regulate the levels of distinct MUP mRNA species.

The major urinary proteins (MUPs) of mice were originally described as a group of antigenically related low-molecular-weight acidic proteins synthesized in the liver, secreted into the serum, and ultimately excreted in the urine (14–16). A homologous protein, α_{2u}-globulin, has been described in rats (24, 31, 32). More recently, hybridization experiments have shown that the MUPs constitute a multigene family of 20 to 30 genes (7, 18). Genetic mapping studies with recombinant inbred mice and mouse-hamster hybrid cell lines indicate that the MUP structural genes are clustered on chromosome 4 (5, 7, 20).

Although the liver appears to be the major site of MUP synthesis, recent studies have shown that the MUP gene family is expressed in several secretory tissues of mice (18, 34). The tissues expressing MUP mRNA sequences are the liver, the submaxillary gland, the lachrymal gland, and the mammary gland. These studies also indicated that expression of MUP mRNA in each of the tissues is under different developmental and hormonal controls (34).

Early studies on the regulation of the MUPs examined the urinary levels of those proteins whose major site of synthesis appears to be the liver (36; this paper). Parfentjer (28) demonstrated that male mice exhibit high levels of proteinuria and that the onset of this proteinuria correlated with the onset of sexual maturity. The role of the major male sex steroid, testosterone, was further indicated by demonstrating that either females or castrated males treated with testosterone attain normal male levels of proteinuria (38). Biochemical examination of these urinary proteins by Finlayson et al. (16) revealed a group of low-molecular-weight acidic proteins which exhibit both strain- and sex-specific electrophoretically distinguishable phenotypes (14, 37). Interestingly, these studies also showed that the administration of testosterone to females resulted not only in an increased amount of excreted MUP, but a shift to the male phenotype.

Nucleic acid hybridization studies (18) have shown that MUP mRNA is the most abundant mRNA in the male mouse liver, constituting approximately 5% of the total. Livers from females and castrated males have approximately one-fifth of the male MUP mRNA level, which increases to normal male levels upon administration of testosterone. However, there is no information regarding regulation of MUP synthesis by hormones other than testosterone.

The expression of rat liver α_{2u}-globulin (the MUP homolog) is also sex related, but unlike the situation in mice, female rat liver contains virtually no α_{2u}-globulin mRNA (23). Also, α_{2u}-glob-

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ulin liver mRNA levels are known to be subject to multihormonal control (22, 23, 25, 29, 30). As with mice, rat liver α_{2u}-globulin consists of a family of approximately 20 genes (21).

Considering the differences and similarities between MUP in mice and α_{2u}-globulin in rats, we addressed a number of questions concerning the hormonal regulation of MUP in the liver. Are the MUPs subject to multihormonal control? If they are, then it should be possible to determine whether different members of the MUP gene complement expressed in the liver are regulated by different hormones, since the products of different MUP genes can be distinguished. Our results indicate that the MUP mRNA levels in the liver are subject to multihormonal controls and that specific members of the MUP gene family are differentially regulated in the various endocrine states examined.

MATERIALS AND METHODS

Mice and hormone treatments. C57BL/6 mice were purchased from Charles River Breeding Laboratories, Inc. Mice were hypophysectomized by Charles River Breeding Laboratories at 8 to 10 weeks of age, and experiments were initiated 12 to 14 days post-hypophysectomy. Drinking water for hypophysectomized mice was supplemented with 5% glucose. Tfm/Y mice (2, 9) were supplied by The Jackson Laboratories, and *little* mice (4, 13) were obtained from breeder stocks from The Jackson Laboratories. All mice were fed solid pellets of Teklad Mouse Breeder Diet (Teklad Mills) containing 17% minimum crude protein, 4% minimum crude fat, and 2.5% minimum crude fiber. Testosterone was supplied as a 35-mg pellet implanted subcutaneously for at least 10 days. Bovine growth hormone (NIH-GH-B18; 0.81 IU/mg) was obtained through the National Pituitary Agency, National Institute of Arthritis, Metabolism and Digestive Diseases, and reconstituted as described (35). Bovine growth hormone was injected intraperitoneally every 12 h for 5 days at a dose of 100 μg per 20 g of body weight, which was previously shown to be optimal (35). Thyroxine (Sigma Chemical Co.) was reconstituted in 10 mM NaOH–0.9% NaCl and injected intraperitoneally every 24 h at a dose of 5 μg per 20 g of body weight. All animals were sacrificed 6 h after the last hormone injection.

RNA extraction and in vitro translation. Mouse liver RNA was extracted using guanidine-hydrochloride as previously described (18). In vitro translations with [^{35}S]methionine (New England Nuclear Corp.) were performed using a mammalian fractionated cell-free system similar to that originally developed by Schreier and Staehelin (33), with some modifications (8, 19). Reaction mixtures (25 μl) contained 5 μg of total liver RNA as indicated.

Polyacrylamide gel electrophoresis. Analysis of in vitro translation products by sodium dodecyl sulfate gel electrophoresis was carried out as previously described (36). For two-dimensional polyacrylamide gel analysis, in vitro translation products synthesized in the presence of dog pancreatic membranes were treated with 100 μg of RNase A per ml for 15 min at 37°C. Samples were then made 1% Nonidet P-40 (wt/vol), 1% Ampholines (pH 4 to 6) (LKB Instruments Inc.), 2.5% β-mercaptoethanol, and 5 M urea and applied to 5% acrylamide–0.15% bisacrylamide tube gels containing 5% glycerol and 2.4% Ampholines (pH 4 to 6). The gels were run for 1 h at 200 V, 18 h at 500 V, and 1 h at 800 V. The two-dimensional separating gel contained 10% acrylamide and 6 M urea. Other two-dimensional gel procedures are essentially as outlined by O'Farrell (27). The narrow-range isoelectric focusing used in the first dimension gives good resolution of the MUP polypeptides, which have pIs ranging from 4.5 to 4.9. Most other liver RNA translation products are much more basic and did not migrate into the gel with these focusing conditions.

Hybridization techniques. All nucleic acid hybridization experiments were done with an MUP cDNA plasmid (p499) which was isolated from a C57BL/6 liver cDNA library. The p499 plasmid has an insert of approximately 900 base pairs and has been shown to code for MUP mRNA by selective hybridization of liver mRNA and in vitro translation (see Fig. 1 and reference 34) and by DNA sequence analysis (M. Woodworth, N. Kuhn, K. Gross, and W. Held, manuscript in preparation). Hybrid mRNA selection and translation experiments were performed as previously described (3, 34). For Northern blot analysis, 15 μg of total RNA was denatured with methylmercury hydroxide (10 mM) at 37°C for 30 min and loaded onto a 1.4% agarose gel (Seakem) containing 50 mM boric acid, 5 mM $Na_2B_4O_7 \cdot 10H_2O$, 10 mM Na_2SO_4, 1 mM EDTA, and 1 mM iodoacetic acid (pH 8.3). Horizontal gels (21 by 15 by 3 mm) were run at 80 V for 5 h and blotted to diazotized ABM paper (Schleicher and Schuell Co.) as described by Alwine et al. (1). The DBM paper was then prehybridized for 4 h in 4× SSC (1× SSC = 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt solution–1% glycine–3 mM sodium phosphate (pH 6.5)–50 μg of denatured salmon sperm DNA per ml, followed by hybridization for 12 h in 4× SSC–1× Denhardt solution–10% dextran sulfate–3 mM sodium phosphate (pH 6.5)–50 μg of denatured salmon sperm DNA per ml–100 ng or 15 × 10^6 cpm of denatured MUP cDNA clone nick translated according to Maniatis et al. (26). The DBM paper was then washed with 2× SSC–0.1% sodium dodecyl sulfate for 3 h and exposed to Kodak XAR film for 12 h at −70°C. Liquid RNA-DNA hybridization was done as described previously (18). The single-stranded DNA probe complementary to MUP mRNA was prepared from p499 DNA by RNA excess hybridization (3).

RESULTS

In vitro translation of MUP mRNA and analysis of MUP polypeptides. Previous examination of urinary MUPs by polyacrylamide gel electrophoresis (37) or by isoelectrofocusing (17) has revealed considerable charge heterogeneity. We have shown that liver mRNA codes for several MUP precursor polypeptides which exhibit both charge and size heterogeneity. Translation of liver mRNA in the presence of a dog pancreas membrane fraction processes the MUP polypeptides to proteins which have molecular weights,

antigenic specificities, and electrophoretic mobilities similar to those of the urinary MUPs (36). These results indicate that the mouse liver contains several MUP mRNAs, which give rise to the different MUP polypeptides.

A number of laboratories have isolated cDNA clones corresponding to MUP mRNAs (3, 10, 12; Woodworth et al., in preparation). The cDNA clone used in these experiments (p499) was constructed from male C57BL/6 liver mRNA and contains an insert of approximately 900 nucleotides. Hybrid mRNA selection and translation followed by two-dimensional gel electrophoresis were utilized to determine whether a single MUP cDNA clone (p499) exhibits sequence homology with all MUP mRNAs present in the liver.

In these experiments, in vitro translation was performed in the presence of dog pancreas membranes to effect processing of the MUP polypeptides. This allowed us to compare the in vitro translation pattern with the stained urinary protein pattern and gave better resolution than two-dimensional gel analysis of precursor MUP polypeptides. p499-selected mRNA translation products were virtually identical to the pattern obtained using total male liver RNA (Fig. 1). Due to the very acidic isoelectric points of the MUP polypeptides (pI 4.5 to 4.8) and the narrow-range isoelectrofocusing in the first dimension (see above), it is possible to directly examine the complement of MUP polypeptides by using total liver translation products. Also, female liver RNA produced a distinctly different pattern of MUP polypeptides, which appears to be a subset of the male pattern (Fig. 1; see also reference 37).

Analysis of the stained urinary MUP polypeptides by two-dimensional gel electrophoresis (Fig. 2) indicated that most urinary MUPs comigrate with processed in vitro synthesized MUPs. Two exceptions were noted. The lower-molecular-weight urinary MUP (Fig. 2, MUP 7), which appeared to exhibit size heterogeneity (doublet), exhibited charge heterogeneity in the in vitro synthesized pattern. The higher-molecular-weight in vitro translation products (Fig. 2, MUP 6), which exhibited both charge and size heterogeneity, were absent from the urinary pattern. This may be due to post-translational modification of these MUPs which occurs in vivo but not in vitro. The more acidic and higher-molecular-weight proteins in the urinary pattern (Fig. 2C) may correspond to these proteins. Only the major MUP spots are labeled in Fig. 2. Some additional spots were present in both the urinary pattern and the in vitro translation pattern. For example, whenever there was a strong MUP 2 or MUP 3 spot, or both, minor spots with a similar charge were apparent at a molecular weight of approximately 17,000 (Fig. 1 and 2C).

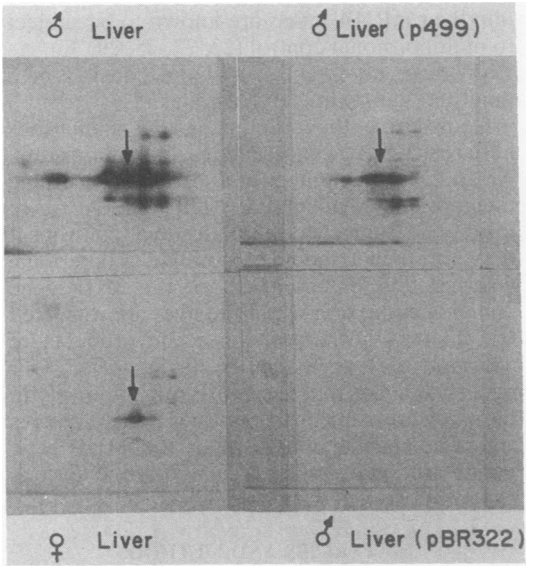

FIG. 1. Two-dimensional polyacrylamide gel analysis of MUP in vitro translation products. Two-dimensional polyacrylamide gel analysis was performed as described in the text. For total male and female liver RNA translation products, approximately 10 μl of the in vitro translation reaction (2×10^6 cpm) was analyzed. The autoradiographs were exposed for 3 days. For the hybrid mRNA selection, 20 μg of total male liver RNA was hybridized to 10 μg of p499 or pBR322 plasmid DNA bound to a nitrocellulose filter. After hybridization and washing, the hybridized RNA was eluted by boiling the filter in water for 90 s, precipitated with ethanol, and translated in a 25-μl in vitro translation reaction, and the products were analyzed. The autoradiograph was exposed for 1 week. Only the acidic (pH 4.5 to 5.0) MUP region of the gel is shown here and in the other figures. The arrows indicate the position of MUP 3 (see Fig. 2). It should be noted that the position of MUP 1 relative to MUP 2 is somewhat variable.

These results establish that the MUP cDNA clone (p499) hybridizes to virtually all liver MUP mRNA (see also reference 34). In addition, two-dimensional gel electrophoresis of in vitro translation products can be used to examine the pattern of MUP mRNAs present in different liver RNA preparations.

Hormonal regulation of MUP mRNA levels. We have utilized a variety of surgically altered and mutant mice to determine whether hormones other than testosterone are involved in controlling MUP mRNA levels. MUP mRNA levels in the livers of mice in various endocrine states were examined by two methods: in vitro translation and RNA:DNA hybridization analysis with the MUP-specific cDNA clone. Al-

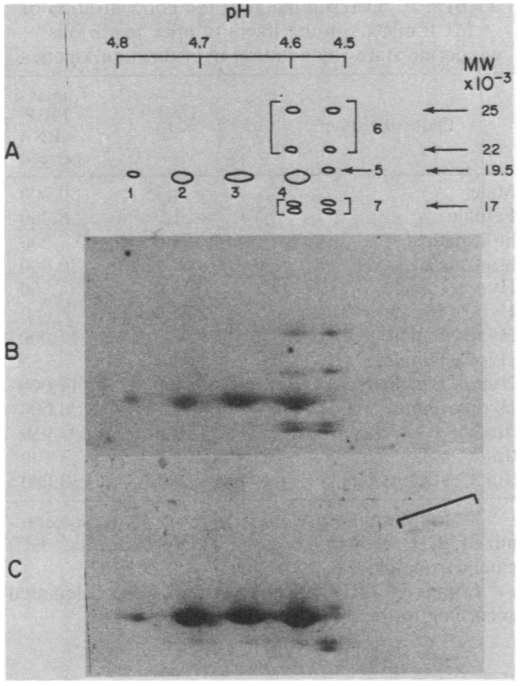

FIG. 2. Two-dimensional polyacrylamide gel analysis of male C57BL/6 MUPs and in vitro translation products directed by male C57BL/6 liver RNA. Approximately 2×10^6 cpm of male C57BL/6 liver RNA translation products was mixed with male C57BL/6 urine (20 μg of protein) and analyzed by two-dimensional polyacrylamide gel electrophoresis as detailed in the text. After electrophoresis, the gel was stained with Coomassie brilliant blue R and photographed (C). The stained gel was then dried and autoradiographed; the exposure was 2 weeks (B). (A) shows a diagrammatic representation of the MUP in vitro translation products. The bar (C) indicates three acidic, higher-molecular-weight proteins present in urine but absent in liver translation products.

though the in vitro translation analysis is not as sensitive or quantitative as the hybridization analysis, it allows indirect examination of the relative levels of several abundant mRNAs simultaneously.

Growth hormone has been implicated in playing a role in several putatively testosterone-controlled genes, including β-glucuronidase in the mouse kidney (35) and α_{2u}-globulin in the rat liver (22, 25, 29, 30). To examine the effect of growth hormone on MUP mRNA levels, we utilized the mutant mouse strain *little* (4, 13). This mutant has a relatively specific pituitary defect resulting in undetectable levels of growth hormone in the serum (W. G. Beamer, personal communication). Figure 3 is an autoradiogram of a sodium dodecyl sulfate gel showing the [^{35}S]methionine-labeled in vitro translation products programmed by RNA derived from the livers of mice in the various endocrine states noted. RNA derived from male *little* mice failed to direct the synthesis of any detectable MUP. However, RNA derived from the livers of male *little* mice treated with growth hormone directed the synthesis of normal male levels of MUP. It should be noted that growth hormone treatment of *little* mice affected the levels of only one other abundant liver mRNA, which codes for a polypeptide of approximately 50,000 daltons (Fig. 3). The lanes which show testosterone-treated female and male liver RNA-directed translation products are presented for comparative purposes.

A direct examination of MUP mRNA levels is shown in Fig. 4. The same RNA preparations used in the translational analysis were analyzed by Northern blot analysis (1). The MUP mRNA levels are extremely low in male *little* mice; however, treatment of male *little* mice with growth hormone fully restored MUP mRNA to

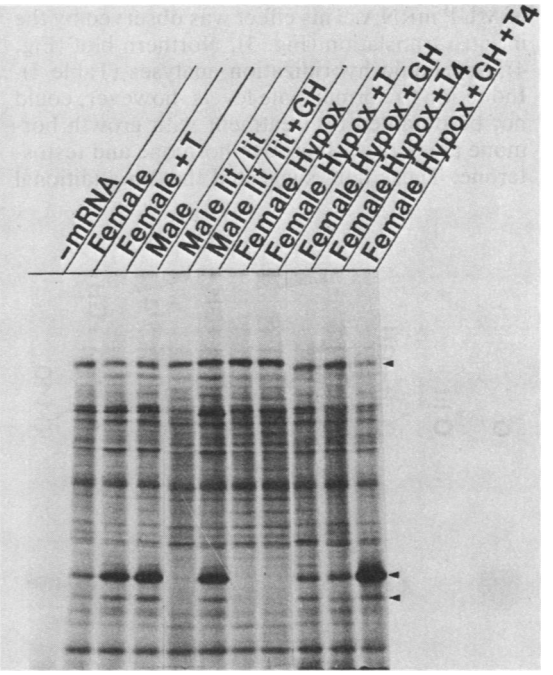

FIG. 3. In vitro translation of liver RNA from mice in various endocrine states. An autoradiogram of a sodium dodecyl sulfate-urea 12.5% polyacrylamide gel of the in vitro translation products programmed by RNA extracted from the livers of mice in various endocrine states is shown. lit/lit, mutant *little* mice, deficient in growth hormone; hypox, hypophysectomized mice; T, testosterone; GH, growth hormone, T4, thyroxine. The two lower arrows indicate the position of the major precursor MUP polypeptides (M_r, 21,500 and 19,500); the upper arrow indicates the position of the albumin precursor (M_r, 70,000).

normal male levels. A more precise quantitation of the absolute amounts of MUP mRNA present was obtained by RNA excess hybridization to a ^{32}P-labeled, single-stranded probe prepared from the MUP cDNA clone p499. Table 1 shows that the MUP mRNA levels in *little* mice are reduced about 150-fold relative to normal adult males.

To determine if growth hormone in the absence of other pituitary-derived or pituitary-controlled hormones was capable of inducing MUP mRNA, we utilized hypophysectomized mice. No MUP mRNA was detected in either hypophysectomized female liver RNA or RNA extracted from the livers of hypophysectomized female mice treated with testosterone (Fig. 3 and 4). Liquid hybridization analysis indicated MUP mRNA levels in hypophysectomized females were reduced approximately 500-fold relative to normal males and that testosterone treatment of hypophysectomized females had no effect on MUP mRNA levels (Table 1). Treatment of hypophysectomized females with growth hormone, however, resulted in a 150-fold increase of MUP mRNA. This effect was observed by the in vitro translation (Fig. 3), Northern blot (Fig. 4), and liquid hybridization analyses (Table 1). Induction to normal male levels, however, could not be obtained by treatment with growth hormone alone or with growth hormone and testosterone. These data suggested that an additional hormone(s) which is present in *little* mice, but absent in hypophysectomized mice, was necessary for complete induction. Therefore, we examined the effects of two hormones known to affect liver metabolism, glucocorticoids and thyroxine. Glucocorticoid treatment of hypophysectomized females resulted in a slight decrease of the already markedly reduced levels of MUP mRNA (Table 1). Thyroxine treatment of hypophysectomized females, however, was found to increase MUP mRNA levels 150-fold (Fig. 3 and 4; Table 1). Treatment of hypophysectomized females with both growth hormone and thyroxine increased MUP mRNA levels approximately 1,000-fold to a level 3-fold higher than that found in normal males (Fig. 3 and 4; Table 1). Administration to hypophysectomized females of testosterone along with growth hormone and thyroxine did not have any additional effect on MUP mRNA levels (data not shown).

We were unable to detect any effect of testosterone on MUP mRNA levels in hypophysectomized mice. However, an approximately 1,000-fold testosterone-independent induction of MUP mRNA was observed when hypophysectomized female mice were treated with both growth hormone and thyroxine. Testicular feminized mice, which are relatively androgen insensitive due to the lack of a functional major testosterone receptor protein (2, 9), were used to further test the testosterone-independent induction of MUP mRNA by growth hormone and thyroxine. Testosterone treatment of tfm/Y mice resulted in only a small (fourfold) increase in MUP mRNA

TABLE 1. Determination of the concentration of MUP mRNA in the livers of mice in various endocrine states by solution hybridization kinetics

Endocrine state[a]	$R_0t_{1/2}$	Copies of MUP mRNA per cell[b]
Male	0.35	30,000
Female	1.7	6,200
lit/lit male	53.0	200
lit/lit male + GH	0.35	30,000
Hypox female	175.0	60
Hypox female + T	175.0	60
Hypox female + GH	1.0	11,000
Hypox female + GC	300.0	35
Hypox female + T4	1.0	11,000
Hypox female + GH + T4	0.12	90,000
tfm/Y	6.0	950
tfm/Y + T	1.5	3,800
tfm/Y + T4 + GH	0.35	30,000

[a] lit/lit, Mutant *little* mice; hypox, hypophysectomized; GH, growth hormone; T, testosterone; GC, glucocorticoids; T4, thyroxine.

[b] Copies of MUP mRNA per cell were calculated according to the method of Hastie et al. (18).

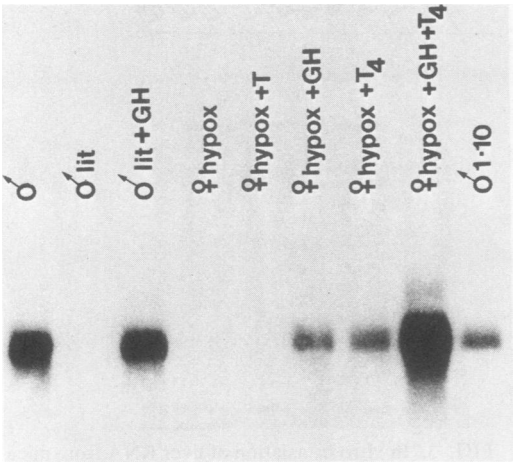

FIG. 4. Northern blot analysis of MUP mRNA levels in the livers of mice in various endocrine states. Fifteen micrograms of total liver RNA was analyzed as described in the text. To provide an estimate of the MUP mRNA levels, a 1:10 dilution of the male liver RNA preparation has been included: 1.5 μg of male liver RNA along with 13.5 μg of carrier male kidney RNA (no MUP mRNA sequences can be detected in kidney RNA). Abbreviations are the same as in the legend to Fig. 3.

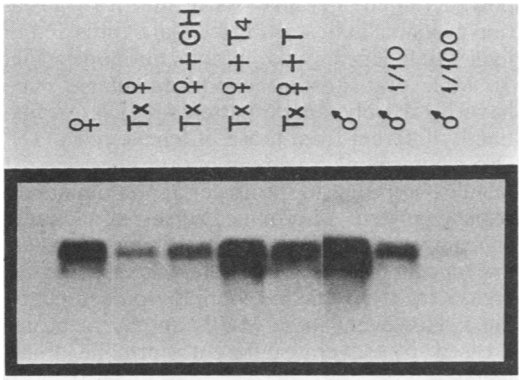

FIG. 5. Northern blot analysis of MUP mRNA levels in hormone-treated thyroidectomized mice. Twenty micrograms of total liver RNA was analyzed on a 1.5% agarose gel containing formaldehyde, blotted, and hybridized to nick-translated p499 plasmid DNA as previously described (34). Female C57BL/6 thyroidectomized mice (Tx) were obtained from Charles River Breeding Laboratories and were treated with the indicated hormone as described in the text.

levels. Treatment of tfm/Y mice with both growth hormone and thyroxine, however, resulted in over a 30-fold increase in the levels of MUP mRNA (Table 1).

Differential regulation of liver MUP mRNA. The occurrence of multiple MUP genes, multiple MUP mRNAs in the liver, and multiple hormones effecting changes in the levels of MUP mRNA raised the question of whether the different members of the MUP gene complement expressed in liver are subject to different hormonal controls. The MUP cDNA clone used in these studies (p499) appears to have considerable homology to all MUP mRNAs expressed in the liver. Thus, possible differential effects cannot be assessed at the nucleic acid level. We have already shown that the male pattern of MUP polypeptides synthesized in vitro differs considerably from the female pattern (Fig.1). Furthermore, testosterone treatment of female mice causes both an increase in MUP mRNA levels and a male pattern of urinary MUPs (37). These results indicate that testosterone has a differential effect on MUP gene expression in the liver and that analysis of the MUP in vitro translation products might provide a more suitable assay to examine differential hormonal effects.

Thyroidectomized female mice were used to examine differential hormonal effects since administration of each of the hormones (testosterone, growth hormone, or thyroxine) results in increased MUP mRNA levels in the liver. Northern gel analysis indicates that thyroidectomized female C57BL/6 mice have approximately one-tenth as much MUP mRNA in the liver as normal females. Administration of growth hormone, testosterone, or thyroxine increased MUP mRNA levels approximately 4-fold, 10-fold, and 30-fold, respectively (Fig. 5). To determine whether a particular hormone influences the level of liver mRNA encoding a particular MUP polypeptide, these RNAs were translated in vitro, and the products were analyzed by two-dimensional polyacrylamide gel electrophoresis (Fig. 6). Growth hormone-treated, thyroidectomized female mice had a pattern very similar to normal females (MUPs 3, 6, and 7). Thyroxine

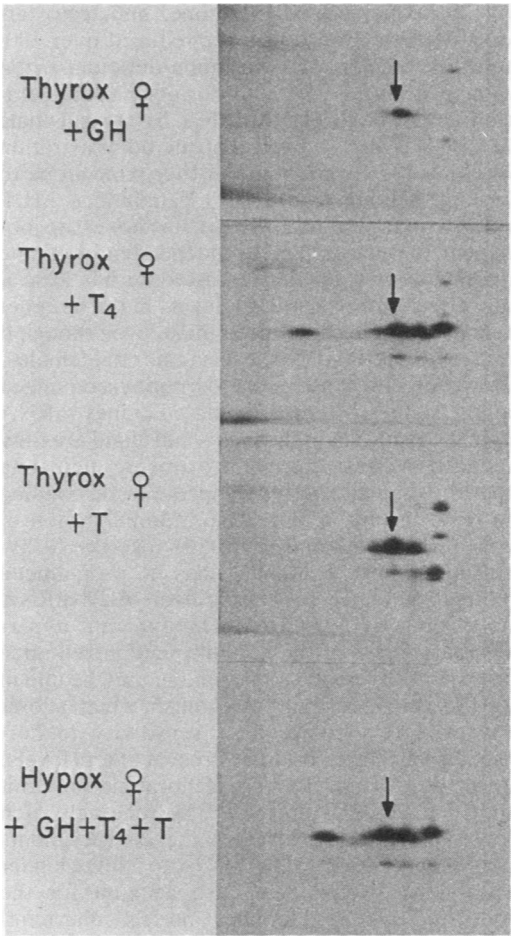

FIG. 6. Two-dimensional polyacrylamide gel analysis of MUP in vitro translation products directed by liver RNA from thyroidectomized female mice treated with growth hormone, thyroxine, or testosterone. The same RNAs analyzed by Northern blot (Fig. 5) were translated in vitro as described in the text. Two-dimensional polyacrylamide gel analysis was performed on 2×10^6 cpm of in vitro translation products. Autoradiographs were exposed for 1 week. Thyrox, thyrodectomized; other abbreviations are the same as in the legend to Fig. 3. Arrows indicate the position of MUP 3 (see Fig. 2).

treatment resulted in a pronounced increase in MUPs 1, 4, and 5, and testosterone treatment increased MUPs 2, 4, 6, and 7. It is clear that mice in different endocrine states exhibit distinct differences in their two-dimensional gel pattern of MUP in vitro translation products.

DISCUSSION

Multihormonal regulation of liver MUP mRNA. The MUPs were previously thought to be the gene products of an androgen-regulated multigene family. In the results presented here, we showed that the liver mRNA levels for the mouse MUPs are under multihormonal control by thyroxine, growth hormone, and testosterone. MUP mRNA levels are reduced over 100-fold in the growth hormone-deficient *little* mouse strain, and growth hormone administration can restore MUP mRNA to normal male levels. We also showed that the administration of either growth hormone or thyroxine alone to hypophysectomized females can induce MUP mRNA over 100-fold. These hormones do not appear to act completely independently of one another. For example, testosterone has little if any effect on MUP mRNA levels in the livers of female hypophysectomized mice, even though it increases MUP mRNA levels in normal females. However, in these same hypophysectomized animals, a testosterone-regulated kidney mRNA and MUP mRNA in the lachrymal gland are fully induced by testosterone treatment, indicating that the animals are fully capable of responding to testosterone (6, 34). Also, administration of growth hormone and thyroxine together to hypophysectomized animals has a pronounced synergistic effect, increasing liver MUP mRNA approximately 1,000-fold. The apparent hyperresponsiveness of the hypophysectomized mice to growth hormone and thyroxine may be due in part to a compensatory mechanism which allows the mice to increase their sensitivity to hormones which are absent or present at low levels. Also, physiological levels of hormone may not fully induce MUP mRNA. The relatively high levels of hormones used in these experiments may thus increase MUP mRNA to a level higher than normal. This may also account for the induction of normal females and testicular feminized males by growth hormone and thyroxine. The experiments presented here do not establish whether these hormones act directly on liver cells. Attempts to demonstrate hormonal regulation of MUP mRNA in primary cultures of isolated hepatocytes have thus far been negative (K. Gaines, H. Baumann, and W. Held, unpublished data).

Differential regulation of liver MUP mRNA. An examination of the MUP proteins expressed in various endocrine states by two-dimensional polyacrylamide gel analysis of in vitro translation products indicates differential expression of liver MUP genes by the various hormones (Fig. 5). Male or testosterone-induced female mice have MUP polypeptide patterns which are distinctly different from those of female mice (37). Growth hormone treatment of thyroidectomized females appears to produce a predominantly female pattern. Thyroxine causes a relatively specific increase in MUPs 1 and 5, and testosterone appears to cause a relatively specific increase in MUPs 6 and 7 in thyroidectomized mice. However, some MUPs appear to be increased by several different hormone treatments.

It should be noted that whereas the treatment of hypophysectomized females with growth hormone, thyroxine, and testosterone increased MUP mRNA approximately 1,000-fold, the two-dimensional polyacrylamide gel pattern of the in vitro translation products was different from the normal male pattern (Fig. 1 and 6). Similar results were obtained with male hypophysectomized mice. Although the reasons for this difference is not entirely clear at this time, it is apparent that some MUP mRNAs are not fully induced in normal mice (i.e., thyroxine induction of MUPs 1 and 5). Also, it is possible that there are additional hormones involved in regulating the MUP mRNA levels.

Regulation of MUP mRNA in other tissues. The hormonal regulation of MUP mRNA levels in the liver is distinctly different from that in other MUP-expressing tissues. Hormones appear to have no positive effect on MUP mRNA levels in the submaxillary gland. Hormonal regulation of MUP mRNA in the mammary gland has not been examined in detail, although by inference the tissue is regulated differently. Interestingly, MUP mRNA in both the liver and the lachrymal gland is subject to regulation by testosterone, but the mode of regulation in each appears to be distinct. Lachrymal MUP mRNAs are regulated by testosterone in the absence of a pituitary gland (34), whereas regulation in the liver by testosterone requires a functional pituitary gland. Presently, it is not clear whether this difference is due to tissue effects or gene-specific effects. Due to large differences between liver and lachrymal MUP mRNA translation products, it is quite likely that they represent transcripts of different MUP genes (34).

Comparison with rat α_{2u}-globulin. The MUPs of the mouse are homologous to the rat urinary protein, α_{2u}-globulin (18). α_{2u}-Globulin is also encoded by a multigene family of approximately 20 genes (21) and has been shown to be subject to multihormonal regulation in the liver by growth hormone, thyroxine, testosterone, and glucocorticoid hormones (22, 23, 25, 29, 30).

There are a number of apparent differences in hormonal regulation between the murine and rat proteins. It is not clear whether α_{2u}-globulin is subject to differential hormonal regulation, as indicated by the results presented here. Chatterjee et al. (11) have concluded that thyroxine indirectly influences α_{2u}-globulin mRNA levels through its effect on pituitary growth hormone. In the results presented here, growth hormone does not fully induce MUP mRNA levels in hypophysectomized mice and appears to affect a subset of MUP mRNA translation products which are somewhat distinct from those affected by thyroxine. In addition, α_{2u}-globulin mRNA levels are affected by glucocorticoid hormone (23, 30), and female rat liver has virtually no α_{2u}-globulin mRNA (23). Although MUP mRNA levels do not appear to be affected by glucocorticoid hormones in C57BL/6 mice, we have observed regulation of MUP mRNA by dexamethasone in other inbred strains. We have also found that females of some species of wild mice have little or no MUP mRNA in the liver (J. Knopf, N. Kuhn, J. Latimer, and W. Held, unpublished data). Thus, some of the apparent differences in regulation may be due to genetic variation within the multigene family. We are currently investigating the genetic basis for these differences.

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LITERATURE CITED

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