Androgen Regulation of MAK mRNAs in Mouse Kidney*

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Androgen-regulated changes in the abundance of two mRNAs in mouse kidney have been studied with the aid of a cDNA clone. The clone was isolated from a library prepared using poly(A)+ RNA from kidneys of androgen-induced mice and is designated MAK-I. Unique DNA sequence from MAK-I hybridizes to multiple RNAs which are approximately 1600 and 2200 nucleotides long, and Southern blot analysis of genomic DNA suggests that there is a single gene from which the RNAs are transcribed. When the MAK RNAs are isolated by hybrid selection and translated in a cell-free system, one polypeptide of 42,000 daltons is obtained. The transcripts, in combination, comprise approximately 0.2% of poly(A)+ RNA in the kidneys of male mice. Following treatment with testosterone, this level increases 3-fold. The uninduced level of MAK mRNA in female kidneys is half of that exhibited by males and is increased 8-fold by testosterone. The mRNAs are also present and their abundance is hormonally controlled in the liver. Levels of MAK RNA detected in submaxillary gland, heart, brain, muscle, and testes are unaffected by testosterone treatment. Differences in the relative amounts of the different sized mRNAs are observed for several tissues. In addition, brain and muscle contain an intermediate sized mRNA instead of the smaller one seen in other tissues. Functional androgen receptor is required for expression of MAK RNA in kidney as shown by reduced levels in Tfm mice. Hormones produced by or controlled by the pituitary gland do not appear to be involved in the regulation of MAK RNA levels.

Because steroid hormone regulation of gene expression has profound effects on mammalian development and physiology, considerable attention has been given to understanding the molecular basis for hormone action. Hormones enter target cells by diffusion, associate with specific cytoplasmic receptors, and are translocated to the nucleus where the hormone-receptor complex binds to several nuclear components, including chromatin. The principle mechanism by which gene expression is modulated is thought to involve increased transcription as a result of high affinity binding of the hormone-receptor complex near the regulated gene. Evidence which supports this model has been accumulating for various classes of steroid hormones, especially glucocorticoids (1-3), progesterone (4), and estrogen (5). Transcriptional regulation of specific genes (6-8) and interaction of the hormone-receptor complex with specific DNA sequences in and near induced genes (9-13) has been reported.

The molecular mechanisms by which androgens affect gene expression, however, are not as well characterized as other hormone classes. Work on androgens has focused primarily on the biological parameters of hormonal control and on quantitation of protein and mRNA levels. Two androgen-stimulated genes, α-globulin which is expressed in liver (14) and prostatic binding protein C3 gene (15), have been transformed into cultured cells, and the latter is responsive to androgens in vitro. In addition, androgens have been shown to regulate transcriptional activity of the C3 gene (16).

Several features of androgen action in kidney are important in making this tissue valuable for examining hormone-regulated gene expression. In contrast to male reproductive tissues such as prostate and seminal vesicle, there is a relative lack of DNA synthesis and cellular hyperplasia in androgen-stimulated kidney (17). Therefore, a stable cell population can be examined. Also, since 5α-reductase levels are low in kidney (18), evaluation of the hormone response is not complicated by conversion of testosterone to 5α-dihydrotestosterone or other metabolic products. Additional aspects of the kidney response to androgen stimulation have been studied and contribute to an overall understanding of hormone action in that tissue. Administration of testosterone to male or female mice results in cellular hypertrophy, increased RNA polymerase activity (19), and increases in RNA and protein synthesis (20). Specific enzyme activities known to increase in kidney following testosterone treatment are β-glucuronidase (21), alcohol dehydrogenase (22), arginase (23), and ornithine decarboxylase (24). Levels of mRNAs represented by cDNA clones KAP (25) and pMK908 (26) are also regulated by androgens.

We have isolated from a mouse kidney cDNA library a clone which corresponds to multiple mRNAs which are regulated by testosterone in kidney and liver and are expressed in numerous tissues. To examine the molecular basis for this hormonal regulation, we have characterized the expression of the RNAs in several tissues and under various hormonal stimuli. We have also examined the protein product of the mRNA by in vitro translation and the gene from which it is transcribed by Southern blot analysis of genomic DNA.

**MATERIALS AND METHODS**

*Animals—Male and female C57BL/6j mice, Tfm/Y, and C57BL/6j-lit mice were purchased from The Jackson Laboratories at 8-10 weeks of age. Hypophysectomised C57BL/6j mice of the same age were purchased from Charles River Mouse Farms. Testosterone inductions were performed by subcutaneous implantation of 30-mg testosterone pellets (a gift of Dr. Roger Ganschow, Children's Hospital Research Foundation, Cincinnati, OH). Unless indicated otherwise, inductions were always for 2 weeks. Hypophysectomised mice were induced 2 weeks after removal of the pituitary gland, and castrated animals were induced 2 weeks after surgery. In some exper-

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Materials—Restriction enzymes were purchased from New England Biolabs, Bethesda Research Laboratories, or Boehringer-Mannheim and used according to manufacturer's instructions. DNA polymerase I was purchased from New England Nuclear, nuclease from Schleicher and Schuell, guanidine hydrochloride (ultrapure) from Schwarz/Mann, and oligo(dT)-cellulose type III from Collaborative Research. Rabbit reticulocyte lysate and [35S]methionine used in *in vitro* translation were purchased from New England Nuclear and Land Biolabs, Bethesda Research Laboratories, or Boehringer-Mannheim and used according to manufacturer's instructions. DNA polymorphism detection reagents were purchased from New England Nuclear, nitrocellulose from Schleicher and Schuell, and autoradiography films were purchased from Eastman. Phosphatase (3000 Ci/mmol) to achieve specific activities of 1-5 x 10^8 cpm/μg. Plasmid DNA used for restriction enzyme analysis was prepared by the rapid boiling method of Hohnes and Quigley (32) or by the following Triton-lysis procedure for restriction fragment isolation and purification. 500 micrograms of whole media containing 1% of tetracycline/ml was inoculated with 0.5 ml of overnight culture, grown to A_{660} of 0.5 and amplified overnight with 16 mg of chloramphenicol/ml of culture. Cells were pelleted the next day by centrifugation at 3000 × g for 20 min at 4°C, washed with 50 mM Tris, pH 8.0, 90 mM NaCl, 50 mM EDTA, and pelleted again. The cell pellet was resuspended in 5 ml of 25% sucrose, 50 mM Tris, pH 8.0, EDTA was added to 50 mM, and the cells were then treated with a solution (0.5/5 ml of 50 mM resuspended pellet) containing 10 mg of lysozyme/ml in 25% sucrose, 50 mM Tris, pH 8.0, for 20 min on ice. An equal volume of the following buffer was added, and the resulting mixture was incubated on ice for 20 min: 50 mM Tris, pH 8.0, 62 mM EDTA, and 0.4% Triton X-100. Following centrifugation at 8000 × g, the supernatant was removed, and closed circular DNA was purified on a cesium chloride-ethidium bromide gradient (33).

Nucleic Acid Isolation—Isolation of total cellular RNA was performed by the method described by Chirgwin et al. (35) which was provided by Dr. Craig Duncan, Children's Hospital Research Foundation, Cincinnati, OH. Freshly excised tissue was homogenized in guanidine hydrochloride solution (34) using a Brinkmann Polytron. Following centrifugation of the homogenate at 12,000 × g for 10 min at 10°C, the supernatant was collected and acidified by addition of 0.25 ml of acetic acid/10 ml. One-half volume of absolute ethanol was added and precipitation carried out for 2 h at −20°C. The resulting precipitate was collected by centrifugation at 12,000 × g for 10 min at 10°C, resuspended in 5-10 ml of guanidine by hydrochloride solution, and precipitated again. The precipitate was collected by centrifugation, resuspended in 10 ml of 0.5% Sarkosyl, 100 μg/ml proteinase K, and incubated at 37°C for at least 1 h. Two phenol and one chloroform extractions were performed. RNA was precipitated overnight by addition of 0.10 volume of 2 M potassium acetate and 2 volumes of absolute ethanol. RNA from the final precipitation was resuspended in autoclaved water, quantitated, and stored at −70°C. An aliquot of each sample was electrophoresed on an agarose gel, and the 18 and 28 S ribosomal RNA bands were excised, eluted in 0.3 M sodium acetate and 2 volumes of absolute ethanol. Polyadenylated RNA was isolated according to Aviv and Leder (35) by oligo(dT)-cellulose chromatography. The method of Blin and Stafford (36) was used as described by Maniatis et al. (33) for isolation of high molecular weight DNA from mouse liver.

Electrophoresis of glyoxalated total or poly(A) RNA was performed on agarose gels to determine the size of the RNA. Polyadenylated RNA was isolated according to Aviv and Leder (35) by oligo(dT)-cellulose chromatography. The method of Aviv and Leder (35) was used as described by Aviv and Leder (35) for isolation of high molecular weight DNA from mouse liver.

**Electrophoresis of glyoxalated total or poly(A) RNA**—Electrophoresis of glyoxalated total or poly(A) RNA was performed as described by Thomas (37). Hybridi-
Digestions of plasmid DNA. Approximate location of the repetitive Vector sequences are represented by thick lines. Sites for the indicated restriction enzymes were determined by double and single enzyme digestions of plasmid DNA. Approximate location of the repetitive element within the insert was determined by Southern blot hybridization analysis of restriction enzyme digestions using the 6.7-kb EcoRI fragment from λ-M58 as a probe. This is shown by the cross-hatched area on the map.

Analysis of mRNA Which Hybridizes to MAK-I—The size and hormonal regulation of the abundance of the mRNA which is represented by the MAK-I cDNA was investigated by Northern blot analysis (37). First, a fragment of MAK-I which does not contain the repetitive sequence indicated on the restriction map was identified. It extends from the leftmost RsaI site of the insert leftward to the pBR322 RsaI site at position 2282. The fragment was isolated by preparative agarose gel electrophoresis and nick translated for use as a probe. Unless otherwise indicated, this 1850-bp RsaI fragment was used in all experiments involving hybridization of RNA or DNA to the cDNA. Total cellular RNA from mouse kidney was denatured with glyoxal, fractionated on 1% agarose, and hybridized with the cDNA fragment. The probe was found to hybridize to two major size species of RNA, 1600 and 2200 nucleotides long. These are shown in Fig. 2. Ethidium bromide-stained 18 and 28 S ribosomal RNA and 9 S mouse globin RNA bands were used as markers in size determinations. The cDNA is probably a copy of the longer mRNA, which is more likely to have been in the 20 S fraction of poly(A) mRNA from which the library was prepared. Identical bands were seen when Northern blots of polyadenylated RNA selected by oligo(dT)-cellulose chromatography of total kidney RNA were probed with the same fragment (data not shown).

Estimates of abundance of these transcripts in the polyadenylated fraction of mouse kidney RNA were obtained by dot blot analysis (Fig. 3). Dilutions of polyadenylated RNA prepared from kidneys of uninduced male mice were spotted onto nitrocellulose for hybridization with a fragment from MAK-I which does not contain repetitive sequence or the cDNA clone for kidney androgen protein, KAP (obtained from Dr. William Held, Roswell Park Memorial Institute, Buffalo, NY). KAP was used as a quantitation standard for kidney mRNA. Details of the quantitation of MAK mRNA are described in the text.

Fig. 1. Restriction enzyme map of cDNA MAK-I. The bar indicates cDNA sequences inserted into the PstI site of pBR322. Vector sequences are represented by thick lines. Sites for the indicated restriction enzymes were determined by double and single enzyme digestions of plasmid DNA. Approximate location of the repetitive sequence is indicated by the cross-hatched area.

Fig. 2. Northern blot analysis of male and female mouse kidney RNA. Total RNA denatured with glyoxal was electrophoresed through 1% agarose, blotted to nitrocellulose, and probed with a 32P-labeled MAK-I fragment. An autoradiogram of the filter is shown. Lanes, from left to right, contain 10 µg of RNA prepared from kidneys of uninduced female, testosterone induced (T) female, uninduced male, testosterone induced (T) male, castrated male or induced (T) castrated male mice. Positions of ethidium bromide-stained 18 and 28 S ribosomal RNA bands are indicated.

Fig. 3. Estimation of the abundance of MAK mRNA in male mouse kidney. Polyadenylated RNA isolated from kidneys of uninduced male mice was spotted onto nitrocellulose and hybridized with a nick-translated fragment from either of two cDNA clones, MAK-I or KAP. Hybridization to KAP was used as a quantitation standard for kidney mRNA. Details of the quantitation of MAK mRNA are described in the text.

Hormonal Regulation of MAK-I RNA in Mouse Kidney—Since the cDNA library from which our clone was selected was prepared from RNA isolated from androgen-induced mice, the hormone regulation of the levels of the RNA complementary to MAK-I was investigated. Male and female 8-week-old C57BL/6J mice were induced for 2 weeks by subcutaneous implantation of 30-mg testosterone pellets. Additional male animals were castrated and after 2 weeks were either used for RNA isolation or induced with testosterone for 2 weeks as described for normal males. Total cellular RNA
was isolated from the kidneys of animals in each of these experimental groups as well as from uninduced male and female mice. The levels of RNA hybridizable with the cDNA probe were compared qualitatively by Northern blot analysis, and the results are shown in Fig. 2. It is apparent that the level of expression in kidneys of uninduced female mice is lower than that in kidneys of uninduced male animals and that basal levels are increased by testosterone administration to animals of either sex. Castration reduces levels of MAK RNA in kidney, but androgen induction of these animals results in levels comparable to that of induced normal males. It appears that abundance of the two RNA species is coordinate ly regulated by testosterone, since the level of each increases and decreases to the same extent under the various hormone conditions described.

To quantitate the effect of androgen on the abundance of the MAK transcripts in kidney, we used a dot blot assay. Polyadenylated RNA isolated from kidneys of male and female mice, either uninduced or treated with testosterone for 2 weeks, was spotted onto nitrocellulose in 2-fold dilutions. A fragment of the cDNA clone which extends from the leftmost RsaI site of the insert leftward to the BglI site 350 bp away was nick translated and used to probe the dot blots. Following hybridization of the RNA dots with the 32P-labeled fragment and autoradiography, areas of the filter containing individual dots were cut out, and the extent of hybridization was determined by liquid scintillation counting. The results of this analysis are shown in Fig. 4. The data were used to evaluate changes in abundance of MAK mRNA between female uninduced and induced mice and also between male uninduced and induced mice. Two-week induction of female mice (Fig. 4A) results in a 8-fold increase in hybridizable RNA whereas the same treatment of male animals (Fig. 4B) produces a 3-fold increase over uninduced levels. Fig. 4, A and B, represents separate experiments and cannot be used to make comparisons between male and female levels of MAK RNA. Results shown in this figure were corroborated by repetitions of the experiment.

The kinetics of the hormonal regulation of MAK RNA were examined to determine the time course of induction and deinduction. Testosterone pellets were implanted into pairs of female mice at designated time points to give induction periods of 12 h to 14 days. Total cellular RNA prepared from kidneys of the induced animals, as well as from uninduced females, was dotted in duplicate onto nitrocellulose and hybridized with the 1850-bp RsaI fragment from MAK-I. Relative intensities of the resulting hybridization signals as determined by densitometry indicate that elevation of MAK RNA levels has begun by 12 h and is maximal by 36 h. This is shown in Fig. 5. A similar experiment was performed to evaluate the length of time required for MAK RNA abundance to return to the uninduced level after hormone is removed from fully induced females. Animals were induced for 4 days, and testosterone pellets were then removed. At time points from 6 to 42 h after removal, RNA was prepared from kidneys of pairs of the deinduced mice. Densitometry of RNA dot shows that MAK RNA levels decrease rapidly following deinduction and are equivalent to those of uninduced animals by 30 h.

Dot blots were used for greater accuracy in quantitation of induction kinetics. However, Northern blots of the same RNA preparations showed that the time course of induction and deinduction is identical for both sizes of MAK RNA (data not shown). Results described above were reproducible when the induction and deinduction experiments were repeated.

Expression in Tfm Mice—Testicular feminization (Tfm) is an X-linked recessive genetic mutation which results in androgen insensitivity of affected animals. The physiological basis for this insensitivity is reduced amounts of the cytosol receptor for testosterone (45). By comparing levels of basal and induced expression of MAK RNA in Tfm and normal male mice, it is possible to evaluate whether the hormonal regulation of this RNA is mediated specifically by testosterone receptors. As shown in Fig. 6, hybridizable RNA in uninduced Tfm kidney is reduced compared to that of uninduced male kidney. Moreover, treatment with testosterone for 2 weeks does not increase the level of hybridization in kidneys of Tfm mice as it does in normal male mice. Female uninduced kidney was included on this blot for comparison of hybridization levels. The low level of expression seen in Tfm kidney may be due to residual amounts of receptor which are known to be present (46) or may be independent of hormonal influence. It is apparent, however, that androgen receptor is required for induction of MAK RNA levels by testosterone in normal kidney. The receptor appears to be involved in a portion of the basal level of expression in normal kidney since uninduced mice bearing the Tfm mutation have less kidney RNA hybridizable to MAK-I than wild type mice do.

Influence of Other Hormones—Involvement of pituitary hormones in the expression of the genes for several testosterone-insensitive renal enzymes has been reported (24, 47). Full androgen response of the liver (48, 49) and submaxillary gland (50) also requires pituitary hormones. The molecular basis for the interaction of testosterom and pituitary hormones in regulating gene expression is unknown. The effect of various pituitary hormones on the level of MAK RNA in kidneys of uninduced and induced female mice is shown in Fig. 7. In this figure the first three lanes can be compared to each other, and the last eight lanes can be compared to each other. Hypopho- sectomy has no effect on basal or induced levels of kidney MAK expression as shown by the hybridization signals in these Northern blots. Further evidence for the lack of multihormonal influence on MAK RNA levels was obtained by examining the effect on expression of manipulating the levels of individual hormones. These results are also shown in Fig. 7. The mutant mouse, lit/lit, lacks detectable levels of circulating growth hormone (51) yet retains normal regulation of MAK RNA abundance in the presence and absence of exogenous testosterone. Furthermore, we were unable to detect an effect of the thyroid hormone, 3,5,3'-triiodothyronine or the synthetic glucocorticoid, dexamethasone, on MAK RNA levels in male mice. It appears that of the hormones which we have tested, kidney MAK is responsive only to manipulations of testosterone levels.

Tissue Distribution of MAK-I Hybridizing RNA—Total cellular RNA isolated from liver, heart, submaxillary gland, brain, and muscle of induced and uninduced female mice was used to examine tissue distribution of RNA homologous to MAK-I. RNA was also examined from testes of uninduced males. In addition to kidney, the liver and submaxillary gland are known to be androgen responsive in the mouse. Expression and regulation of mRNA which hybridizes to the MAK-I cDNA in those tissues were of particular interest. We also expected these experiments to provide information about testosterone regulation in other tissues and possible tissue-specific expression of one or the other of the two RNA species. Results of Northern blot analysis of these RNA preparations are shown in Figs. 8 and 9. Overexposure for the lanes containing kidney RNA was done in order to visualize bands in lanes containing RNA from other tissues. MAK-I complementary RNA is expressed in every tissue that we have examined. In addition, we have found that the mRNA is
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Polyadenylated RNA [μg]

Female

Polyadenylated RNA [μg]

Male

Fig. 4. Quantitation of the induction of MAK mRNA by testosterone in male and female kidney. Polyadenylated RNA from kidneys of female (A) or male (B) induced and uninduced mice was spotted onto nitrocellulose. Following hybridization with a nick-translated fragment from MAK-I and autoradiography (shown above graphs), the extent of hybridization was determined by liquid scintillation counting. In both A and B, open circles indicate mRNA from uninduced animals, and closed circles indicate mRNA from induced animals.

Fig. 5. Kinetics of testosterone induction and deinduction of MAK RNA in kidney. Total RNA (5 μg) prepared from kidneys of uninduced female mice and females induced with testosterone for 12, 24, and 36 h, and 2, 4, 6, 8, and 14 days was dotted onto nitrocellulose. RNA prepared 12, 18, 24, 30, 36, and 42 h after removal of the testosterone pellet from fully induced mice was also dotted onto nitrocellulose, and both sets of dots were hybridized with a 32P-labeled fragment from MAK-I. Relative intensities of the dots were determined by densitometry and plotted as shown in A. The arrow indicates the time at which deinduction began. In B an autoradiogram of the RNA dots is shown.

Inducible in liver and that an intermediate size species is present in some tissues instead of the smaller mRNA observed in kidney.

Qualitative comparison of the intensity of hybridization signals for kidney RNA and for RNA from other tissues allows us to draw several conclusions. The level of expression of MAK RNA in all other tissues examined is less than that observed for even uninduced female kidney. Submaxillary
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gland, heart, brain, muscle, and induced liver each contain similar amounts of RNA which hybridize with MAK-I. We estimate that there is approximately 4-fold less MAK RNA in these tissues than in female uninduced kidney since similar signal intensities are produced in Northern blots by 5 µg of kidney RNA as 20 µg of muscle or brain RNA (Fig. 9). The pattern of expression in liver is interesting because neither male (not shown) nor female mice have levels of MAK RNA detectable under the conditions of our Northern blots until the animals are induced with testosterone. The low level of basal expression found in submaxillary gland, heart, brain, and muscle, however, is not affected by androgen treatment of the animals. This is shown in Fig. 8 for submaxillary gland and heart and is not shown for brain and muscle. Testes also exhibits detectable levels of MAK RNA (Fig. 9), and testosterone regulation has not been examined.

Variations in the relative levels of abundance of multiple MAK RNAs are evident among the tissues examined. Liver and submaxillary gland, like the kidney, show stronger hybridization signals to the larger of the RNA species. Northern blots of RNA from heart, as shown in Fig. 8, have consistently indicated that the smaller RNA is slightly more abundant than the larger RNA in that tissue. Fig. 9 shows that in mouse brain and muscle an intermediate-sized RNA species is the predominant one expressed. Testes contains RNA species which are similar in size to those of brain and muscle but which are of equal abundance. Differential regulation of the mechanism(s) responsible for generation of multiple RNAs is, therefore, apparent among the tissues examined resulting in various levels of expression of several RNA species.

Translation of mRNA Selected by MAK-I cDNA—The translation product of the mRNA complementary to the cDNA clone was identified by hybrid selection of polyadenylated RNA, in vitro translation, and polyacrylamide gel electrophoresis. The same cDNA fragment used for Northern blot analyses was bound to a nitrocellulose filter and used to capture complementary mRNA from a hybridization solution containing kidney poly(A+) RNA prepared from uninduced or induced animals. The mRNA eluted from the filters was translated in vitro using a reticulocyte lysate cell-free system. Translation products were electrophoresed on polyacrylamide-sodium dodecyl sulfate gels and visualized by autoradiography (Fig. 10). Although the cDNA fragment used for capture hybridizes to the two sizes of poly(A+) RNA seen on a Northern blot, only one translation product was observed. The molecular weight of the polypeptide is 42,000. More of this product is produced by RNA selected from induced kidney than from uninduced kidney, thus establishing a correlation between this polypeptide and regulation of the MAK RNA. Either of the two MAK RNA species is long enough to encode a protein of this size. We do not know whether it is translated from one or both of the mRNAs.

Fig. 6. Expression of MAK mRNA in Tfm mice. Northern blot analysis of 10 µg of glyoxal-denatured total RNA showing relative concentrations of MAK mRNA in kidneys of uninduced males, testosterone induced (T) males, uninduced Tfm/Y, and testosterone induced (T) Tfm/Y mice. RNA from kidneys of uninduced female mice was included for comparison.

Fig. 7. Influence of additional hormones on MAK mRNA expression in kidney. Ten micrograms of total RNA isolated from kidneys of normal female uninduced, normal induced (T), hypophysectomized uninduced (Hpx), hypophysectomized induced (T), lit/lit uninduced (lit/lit), lit/lit induced (T), normal male uninduced, male 3,5,3'-triiodothyronine induced (T3) male dexamethasone induced (dex), and male testosterone induced (T) mice was denatured, electrophoresed, and blotted onto nitrocellulose for hybridization with a nick-translated cDNA fragment. An autoradiogram of the filter is shown. The first three lanes and last eight lanes are results of two separate experiments.
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**FIG. 8.** Distribution and testosterone regulation of MAK mRNA in various mouse tissues. Ten micrograms of total RNA isolated from kidney, liver, submaxillary gland, and heart was denatured with glyoxal, electrophoresed, and blotted for hybridization with a 32P-labeled fragment from MAK-I. RNA was prepared from tissues taken from uninduced female mice and from female mice induced with testosterone as indicated by T. An autoradiogram of the filter is shown.

**FIG. 9.** Expression of additional MAK mRNAs in mouse tissues. Five micrograms of total RNA isolated from kidney and 20 μg of RNA isolated from brain, muscle, or testes were denatured with glyoxal, electrophoresed, and blotted for hybridization as described previously. All RNA was prepared from kidneys of uninduced mice.

**Fig. 10.** In vitro translation of MAK complementary mRNA. The 1850-bp RsaI fragment from MAK-I was bound to nitrocellulose and hybridized with poly(A') RNA isolated from kidneys of uninduced female mice and testosterone-induced female mice. Captured mRNA was eluted and translated in vitro in a rabbit reticulocyte lysate in the presence of [35S]methionine. Translation products were electrophoresed on 10% polyacrylamide gels. An autoradiogram of the gel is shown. Translation reactions contained either no RNA, control kidney mRNA, eluted kidney mRNA, or RNA captured by a filter to which pUC8 was bound. Positions of molecular weight standards and the polypeptide which is translated from MAK RNA are indicated.

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**DISCUSSION**

Androgens are known to affect gene expression in mouse kidney. Examination of the molecular basis for this hormonal regulation requires that specific gene products be identified and their control by androgens characterized. By isolating a cDNA clone, MAK-I, which corresponds to androgen-regulated mRNAs, it has been possible to examine the expression and hormonal regulation of those mRNAs. Results of this study indicate that regulation of the MAK gene is unique among androgen-controlled genes which have been characterized and provides a useful system for studying hormone action.

Genomic Sequences Complementary to MAK-I—The nature of genomic sequences corresponding to the cDNA clone was investigated by hybridization of a nonrepetitive fragment of MAK-I to genomic Southern blots of C57BL/6J mouse DNA digested with EcoRI, BamHI, PvuII, PsI, HindIII, KpnI, or BglII (Fig. 11). The fragment used extends from the rightmost RsaI fragment of the insert rightward to the BglI site 350

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Following administration of hormone, a change in the level of MAK mRNA is evident by 12 h and is maximal by 36 h. By contrast, β-glucuronidase induction has a lag time of 1-4 days (53) and slowly increases to reach steady state levels by 20-30 days. Ornithine decarboxylase activity begins to increase between 24 and 48 h and peaks to maximum induced levels after 5 days (24). Induction of 908 RNA (26) is evident within 4 h and complete by 14 h. Variations in the response time of different genes to a single hormone also exists for other classes of steroids, and the mechanisms responsible for these variations remain to be explained (6). It has been proposed (6,53) that the kinetics of induction reflect the time required to fill multiple hormone receptor-binding sites. Paajanen et al. (54) have demonstrated that the lag time of β-glucuronidase induction can be affected by the nuclear residence time of androgen receptors and suggest that this provides support from the above mentioned proposal. It is also possible that other proteins or mRNAs are induced by testosterone during the lag time and that they represent the primary response to hormone administration. At this time we have no direct evidence that MAK regulation represents a primary response.

Examination of the time course of deinduction indicates that MAK RNA abundance falls rapidly to uninduced levels when the hormone is removed from fully induced female mice. These results are similar to those obtained for β-glucuronidase deinduction (50) and are consistent with rapid reduction of kidney nuclear receptor levels observed (54) following administration of a single dose of testosterone that is comparable to that achieved with pellets.

Induction of MAK mRNA requires a cytoplasmic testosterone receptor as indicated by experiments performed with RNA from testicular feminized mice. Animals carrying the Tfm mutation have reduced amounts of the testosterone receptor and are androgen insensitive (45). Levels of MAK RNA are lower in these mice than in normal males and cannot be increased by administration of testosterone. The requirement for a testosterone receptor indicates that the induction of MAK RNA in mouse kidney is due specifically to testosterone and not of its metabolites. Estradiol and 5α-steroids are metabolic products known to be active in other tissues, but which function through their own receptors. 5α-Dihydrotestosterone is a metabolite which shares the testosterone receptor and, therefore, ineffective in Tfm mice. However, low levels of 5α-reductase in kidney preclude conversion of testosterone to 5α-dihydrotestosterone. Hormonal regulation of MAK gene expression is due specifically to testosterone and is mediated by the testosterone receptor.

MAK mRNA levels are not affected by hormones produced by or regulated by the pituitary gland. This is indicated by the fact that basal and testosterone-induced levels of MAK RNA are not altered by hypophysectomy, absence of growth hormone, or administration of thyroid hormone and corticosteroids. Involvement of pituitary hormones has been implicated in expression of several androgen-regulated genes, and it is clear that this multihormonal control is complex. Hypophysectomy has no effect on basal expression of β-glucuronidase but diminishes the usual testosterone induction by 25% (47). Basal levels of two liver proteins, αα-globulin and MUP, are reduced in the absence of pituitary influence and various combinations of thyroxin, glucocorticoids, growth hormone, and insulin are required in conjunction with testosterone for induction (48, 58). Androgen regulation in the kidney of the 908 RNAs (26), alcohol dehydrogenase, and D-amino oxidase (47) are all independent of the pituitary gland suggesting that there is not a general effect of pituitary

![Fig. 11. Hybridization of MAK-I to mouse genomic DNA.](image)

High molecular weight mouse DNA (10 μg) digested with the indicated restriction enzymes was electrophoresed on 0.8% agarose gels and transferred to Zetapore for hybridization with a 32P-labeled dominant size species of RNA in mouse kidney. They are restriction enzymes was electrophoresed on fragment described in the text. An autoradiogram of the blot is shown. Sizes of ethidium bromide-stained marker fragments are indicated.

A fragment from the MAK-I insert hybridizes to two predominant size species of RNA in mouse kidney. They are approximately 2200 and 1600 bases in length, are present in both female and male kidney, and their abundance is regulated by testosterone. Androgen control is indicated by the lower level of MAK RNA in kidneys of female and castrated mice compared to males and also by the increased expression of MAK mRNA which occurs following exogenous administration of testosterone. From an uninduced level of approximately 0.2% of polyadenylated RNA in male kidney, MAK mRNA increases 3-fold with induction. In female kidney, testosterone increases its abundance 8-fold from an uninduced level of about 0.1% of poly(A+) RNA. The induction of MAK mRNA in kidneys of female mice is comparable to that reported for KAP (25), pMK908 (26), and alcohol dehydrogenase (52), all of which are 8-10 times more abundant in female kidney following testosterone treatment. Changes in enzyme activity have also been described for β-glucuronidase (21) and ornithine decarboxylase (24) which increase 20-50-fold and 100-fold, respectively.

Induction of MAK mRNA in kidney is relatively rapid.
hormones on the kidney testosterone response. The lack of multihormonal control of MAK expression should allow more straightforward analysis of the molecular basis of testosterone regulation.

MAK mRNA is present not only in kidney, but in liver, submaxillary gland, heart, brain, muscle, and testes. The distribution of this mRNA suggests that it may code for a housekeeping function or as in the case of β-glucuronidase is present in many tissues but not inducible in all androgen-responsive ones. The reason for androgen regulation of specific enzymes in murine kidney is unknown. In mouse kidney testosterone does not act to induce differentiation and production of large quantities of tissue-specific proteins in a manner such as estrogen and progesterone affect chick oviduct. By comparing levels of expression in tissues taken from induced and uninduced mice, it was found that the abundance of MAK mRNA is regulated by testosterone in liver as well as in kidney. A third androgen target tissue, submaxillary gland, does not exhibit hormonal control of MAK mRNA. To our knowledge, there is no other example of testosterone regulation of one gene in two different tissues. While levels of MUP and α2-globulin mRNA are androgen controlled in both liver and lachrymal gland (49, 59), it is suspected that different members of their respective gene families are responsible for the observed expression. We do not know to what extent hormonal control of the MAK gene in liver resembles that in kidney. An opportunity may exist to compare the molecular basis for hormone action in two tissues where the MAK gene is regulated differently by androgens.

A fragment from MAK-I was found to hybridize differentially to several RNA species among various tissues which were examined. In Northern blots of kidney, liver, submaxillary gland, and heart RNA two hybridization signals are evident, while in brain and muscle an RNA which migrates between these two is observed instead of the small species. We have no evidence to suggest a correlation between hormonal regulation and appearance of specific mRNAs. Generation of multiple RNAs can result from transsplicing of similar members of a gene family or by differential processing of the transcript from a single gene. There are several mechanisms by which differential processing might occur, and precedent exists for each of them. Use of alternate promoters (60–62) or multiple polyadenylation signals (63–65) results in mRNAs which differ in the length of 5′ or 3′ untranslated regions. These mRNAs code for identical proteins. In several systems (60, 62, 64) tissue or developmental specificity of the various mRNAs produced by these mechanisms has been observed. Differential splicing of exons and synthesis of two polypeptides from one gene has also been demonstrated (66–68). Results of our genomic Southern blot analysis strongly suggest that the MAK RNAs are transcribed from one gene and not from separate members of a gene family. Therefore, differential processing of transcripts or use of multiple promoters seems likely. Since in vitro translation of MAK RNA isolated by hybrid selection produces a single polypeptide, the kidney RNAs do not appear to be generated by differential splicing of coding sequences. Evidence to support this idea comes from preliminary characterization of additional cDNA clones which indicates that alternate polyadenylation signals are used to generate the mRNAs that we observe in kidney. The polypeptide product of the intermediate sized brain and muscle mRNA has not been investigated. Additional information about the molecular basis for the mRNAs being produced from this gene in various tissues will be produced by isolation and characterization of genomic clones and additional cDNA clones for MAK.

Examination of MAK mRNA expression and hormonal regulation of that expression in mouse kidney indicates that this will be a useful system in which to study the molecular basis for androgen action in non-sex tissues. The combination of characteristics described for this gene is unlike that of any other testosterone-inducible gene that has been studied and may allow interesting comparisons to be made. In addition, production of multiple mRNAs from one gene and tissue-specific expression of one of them should enable us to investigate mechanisms which govern mRNA synthesis and processing.

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