Genetic Regulation of Estrogen-Dependent Repression of Female-Specific Testosterone 16α-Hydroxylase (I-P-450$_{16\alpha}$) in Male Mouse Liver: Murine Ripr Locus

Mitsuhide Noshiro and Masahiko Negishi*,‡

Laboratory of Cellular and Molecular Pharmacology, National Institute of Environmental Health Sciences, National Institutes of Health, P.O. Box 12233, Research Triangle Park, North Carolina 27709

Received October 27, 1987; Revised Manuscript Received March 22, 1988

ABSTRACT: The genetic basis for repression of I-P-450$_{16\alpha}$ in livers of male mice was examined in 129/J and BALB/cJ mice. Castration of adult male BALB/cJ but not 129/J mice resulted in derepression of I-P-450$_{16\alpha}$ at its mRNA and activity levels. It was further found that the patterns of derepression in (129/J × BALB/cJ)F1 and F2 offspring indicated that the derepression of I-P-450$_{16\alpha}$ is inherited as an autosomal additive trait. The distribution of derepression among castrated recombinant inbred strains (9×A) indicated a close link of a locus repressing I-P-450$_{16\alpha}$ in male mice to the Rip locus on chromosome 7. Rip was previously defined as a locus that regulates specific expression of I-P-450$_{16\alpha}$ in livers of female mice [Noshiro, M., Lakso, M., Kawajiri, K., & Negishi, M. (1988) *Biochemistry* (preceding paper in this issue)]. Other tested inbred mice (A/HeJ, C57BL/6J, C3H/HeJ, and DBA/2J) showed the derepression of I-P-450$_{16\alpha}$ by castration, such as BALB/cJ. We propose Ripr (*r*epression of an action of *Rip* locus in male mice) as the name of the locus by which repression of I-P-450$_{16\alpha}$ is regulated in male mice. Treatment of castrated male BALB/cJ mice by testosterone propionate, estradiol valerate, or diethylstilbestrol repressed I-P-450$_{16\alpha}$ to the levels seen in normal BALB/cJ male mice. Dihydrotestosterone, however, had little effect in repressing I-P-450$_{16\alpha}$ in castrated mice. The results suggested that estrogen rather than androgen is a repressor of I-P-450$_{16\alpha}$ in livers of male mice. The genetic and hormonal studies indicated that the Ripr locus is responsible for the estrogen-dependent repression of I-P-450$_{16\alpha}$.

Sexual differentiation of drug metabolism and hydroxylation of endogenous compounds such as steroid hormones by the liver microsomal monooxygenase system in rodents has been known for some time. The differentiation of these activities is due to sex-dependent expression of a form of cytochrome P-450 (P-450)[1] specific for the activity in male or female animals. P-450 represents a group of terminal oxidases in the microsomal system. Many proteins and enzymatic activities other than P-450 are known to exhibit sexual dimorphism, including prolactin receptor and mouse major urinary protein (Colby, 1980).

Testosterone 16α-hydroxylase activity in liver microsomes is higher in male than in female 129/J mice, while the activity is equally high in female and male BALB/cJ and C57BL/6J mice (Ford et al., 1979; Harada & Negishi, 1984a; Noshiro et al., 1986). In order to elucidate the molecular mechanism of sex-dependent expression of the 16α-hydroxylase, we purified forms of P-450 specific for 16α-hydroxylation of testosterone from 129/J mice (Harada & Negishi, 1984b; Devore et al., 1985) and demonstrated that at least two isozymes of testosterone 16α-hydroxylase are found in mouse liver microsomes; one (I-P-450$_{16\alpha}$) is expressed specifically in female mice, and the other (C-P-450$_{16\alpha}$) is the major isozyme in male mice (Noshiro et al., 1986; Harada & Negishi, 1985). This female-speciifc I-P-450$_{16\alpha}$ was repressed in 129/J female mice but not in BALB/cJ female mice, resulting in a difference in levels of testosterone 16α-hydroxylase activity at its mRNA levels in females of the two stains (Noshiro et al., 1986). The repression of I-P-450$_{16\alpha}$ in 129/J female mice (or the expression of I-P-450$_{16\alpha}$ in BALB/cJ female mice) is regulated by the Rip locus on chromosome 7 (Noshiro et al., 1988).

The sex-dependent expression and repression of liver proteins and enzymes are believed to be regulated by a complex mechanism involving multiple hormones such as gonadol, pituitary, thyroid, and adrenal hormones. Recently, we demonstrated that growth hormone represses I-P-450$_{16\alpha}$ in male mice, whereas growth hormone is necessary factor for expression of male-specific C-P-450$_{16\alpha}$ in male mice (Noshiro & Negishi, 1986b). It remains to be shown, however, whether hormones other than growth hormones are involved in the repression of I-P-450$_{16\alpha}$ in male mice.

In this report, we have used 129/J and BALB/cJ mice to investigate whether the Rip locus regulates the repression of I-P-450$_{16\alpha}$ in male mice and whether the gonads/hormones are involved in the repression. Genetic and hormonal mechanisms of repression of I-P-450$_{16\alpha}$ in male mice will be discussed.

Experimental Procedures

Animals and Hormone Treatments. In all of the experiments, 6–9-week-old mice were used. All inbred mouse strains were purchased from Jackson Laboratory. F1 and F2 offspring from crosses between the 129/J and BALB/cJ strains were

* Author to whom correspondence should be addressed.
‡ Present address: Pharmacogenetics Section, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, P.O. Box 12233, Research Triangle Park, NC 27709.

[1] Abbreviations: DES, diethylstilbestrol; DHT, dihydrotestosterone; E_2, estradiol valerate; LH, luteinizing hormone; P-450, cytochrome P-450; Rip locus, *r*egulation of sex-dependent, constitutive expression of phenobarbital-*i*nducible *P*-450; Ripr, *r*epression of an action of *Rip* locus in male mice; TP, testosterone propionate.

provided by the Comparative Medicine Branch, National Institute of Environmental Health Sciences (NIEHS). Recombinant inbred strains (9×A) were supplied by Dr. Leroy Stevens (Jackson Laboratory). Male mice were castrated and housed for 2 weeks before hormone treatments began. Sex hormones (testosterone, dihydrotestosterone, and estradiol) and diethylstilbestrol were dissolved in corn oil and administered ip.

Preparation of Microsomes and Total Poly(A+) RNA from Mouse Livers. Mouse livers pooled from three to five mice, except for the experiments with F2 offspring, were minced and divided into two parts. One part of the minced livers was homogenated to prepare liver microsomes according to the procedure described previously (Noshiro et al., 1986). The microsomal pellets obtained were suspended in 100 mM potassium phosphate buffer, pH 7.5, containing 250 mM sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol, and 2 μg/mL of leupeptin. Total RNA was extracted from the remaining minced livers by guanidine hydrochloride as previously described (Cox, 1968), and then poly(A+) RNA was purified from total liver by oligo(dT)–cellulose column chromatography (Aviv & Leder, 1972).

Cloning of Mouse cDNAs for I-P-450$_{16\alpha}$. Liver poly(A+) RNA was purified from untreated BALB/cJ female mice in which I-P-450$_{16\alpha}$ is constitutively expressed. A cDNA library was constructed from liver poly(A+) RNA from BALB/cJ females by using λgt11 as the cloning vector according to the methods of Young and Davis (1982). Twenty-one individual clones were isolated and divided into three groups on the basis of their restriction maps. Complete nucleotide sequences were obtained for the two types of cDNA (pf26 and pf3/46). The cDNA pf26 and pf3/46 share 83% and 93% nucleotide sequence similarity to rat P-450b, respectively. The details of this cloning work are described in Noshiro et al. (1988).

Northern and Dot Hybridizations. Liver poly(A+) RNA was electrophoresed on 1% agarose gel containing 5 mM methylmercury hydroxide (Bailey & Davidson, 1976) and transferred to either DBM paper or nylon paper (Nytran; Schleicher & Schuell). Dot hybridization experiments were performed as described by Norstedt and Palmitter (1984). The hybridization conditions were previously described (Noshiro et al., 1986).

Other Analytical Methods. Testosterone 16α-hydroxylase activity was measured by the methods of Harada and Negishi (1984a). The isozyme-dependent activity was determined as activity inhibited by antibody to I-P-450$_{16\alpha}$ or C-P-450$_{16\alpha}$ as described previously (Noshiro et al., 1986).

Protein contents were measured by using the Bio-Rad protein assay kit according to the method of Bradford (1976).

Results

Effect of Castration on Levels of mRNA and Activity of I-P-450$_{16\alpha}$. As previously reported (Noshiro et al., 1986), I-P-450$_{16\alpha}$ is constitutively a female-specific isozyme of testosterone 16α-hydroxylase and the levels of this isozyme are at least 10 to 20 times lower in male than in female BALB/cJ mice. Testing of eight other inbred mouse strains showed the female-specific expression of the isozyme is a general phenomenon in mouse liver, except in 129/J mice. I-P-450$_{16\alpha}$ is severely repressed not only in males but also in females of 129/J mice.

In order to investigate the effect of sex hormones on the repression of I-P-450$_{16\alpha}$ in male mice, catalytic activity and mRNA levels of I-P-450$_{16\alpha}$ were measured in livers from castrated 129/J, BALB/cJ, and 129CF1/J mice. Liver poly(A+) RNA and microsomes were prepared from sham-operated and castrated mice, and Northern hybridizationn of poly(A+) RNA with ^{32}P-labeled pf26 cDNA and assay of testosterone 16α-hydroxylase activity in liver microsomes were carried out. The results are shown in Figure 1 and Table I. In sham-operated male mice only a small amount of hybridization was detected, which is consistent with previous findings (Noshiro et al., 1986). On the other hand, castration dramatically increased the mRNA levels in BALB/cJ male mice, but had no effect in 129/J male mice. In castrated 129CF1/J mice the hybridizable mRNA was induced to approximately half the level of that found in the castrated BALB/cJ mice. The effects of castration on I-P-450$_{16\alpha}$-dependent activity levels in liver microsomes of these mice coincided with those of the mRNA levels. The results showed a clear strain difference in derepression of I-P-450$_{16\alpha}$ in castrated 129/J and BALB/cJ male mice. Furthermore, the intermediate levels in 129CF1/J offspring were suggestive of an additive inheritance of I-P-450$_{16\alpha}$ repression at mRNA and activity levels in 129/J and BALB/cJ pairs.

Autosomal Additive Inheritance of Derepression of I-P-450$_{16\alpha}$ in Castrated 129/J and BALB/cJ. Liver microsomes and poly(A+) RNA were isolated from individuals of castrated 129CF2/J male offspring. The relative amounts of I-P-450$_{16\alpha}$ mRNA in these mice were estimated by dot hybridization experiments with pf26 cDNA as probe, and the results are

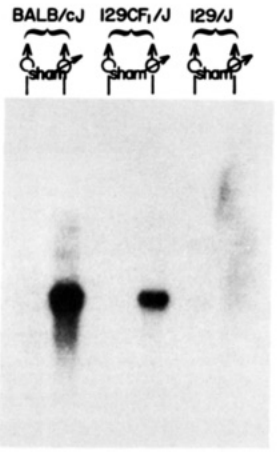

FIGURE 1: Northern hybridization of pf26 cDNA with liver poly(A+) RNA from sham-operated and castrated BALB/cJ, 129CF1/J, and 129/J male mice. Two weeks after the operation, five mice from each strain were killed and five livers were pooled and minced. Poly(A+) RNA was prepared from half of the minced livers, and 2 μg was electrophoresed on agarose gel under the denatured condition described under Experimental Procedures and transferred to DBM paper. The cDNA clone pf26 (1.8-kb insert) was nick-translated with [^{32}P]dCTP, and approximately 1×10^8 cpm of the radioactive cDNA insert was incubated with the DBM paper in the hybridization conditions described (Noshiro et al., 1986). The hybridized DBM paper was washed and exposed to X-ray films.

Table I: Isozyme-Dependent Activities in Liver Microsomes from Castrated Mice[a]

	testosterone 16α-hydroxylase activity [nmol min^{-1} (mg of protein)$^{-1}$]			
	I-P-450$_{16\alpha}$ dependent		C-P-450$_{16\alpha}$ dependent	
	sham operated	castrated	sham operated	castrated
BALB/cJ	0.04	0.39	0.59	0.61
129CF1/J	0.02	0.11		
129/J	0.03	0.01	0.66	0.41

[a] Liver microsomes (200 μg) were used for the assay. Testosterone 16α-hydroxylase activities in the liver microsomes were measured in the presence or absence of specific inhibitory antibodies to I-P-450$_{16\alpha}$ or C-P-450$_{16\alpha}$. The isozyme-specific 16α-hydroxylase activities were determined as the activity inhibitied by the antibodies.

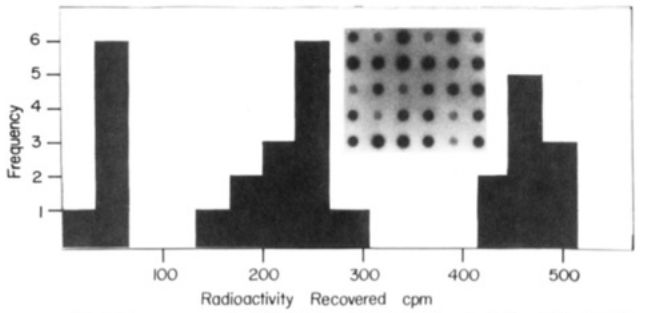

FIGURE 2: Histograms of mRNA levels hybridized with pf26 cDNA in castrated F2 offspring of 129/J × BALB/cJ. Poly(A+) RNA was prepared from 30 castrated 129CF2 offspring as described under Experimental Procedures. A 1-μg aliquot of each poly(A+) RNA was dotted on nitrocellulose paper and hybridized with the ^{32}P-labeled cDNA insert of pf26. After exposure to X-ray, the hybridized areas were cut and their radioactivities measured by scintillation counting. The experiments were repeated 4 times, and average values obtained for each sample were used to make this histogram. The included picture is a typical example for the dot hybridization. The dot hybridization with 1 μg of poly(A+) RNA was in the range that the degree of hybridization in linearly correlated with the amount of poly(A+) RNA dotted as shown previously (Noshiro et al., 1986a).

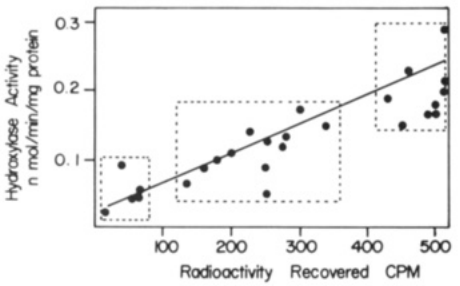

FIGURE 3: Linear correlation between I-P-450$_{16\alpha}$-dependent activity and mRNA levels in castrated 129CF2 offspring. Liver microsomes were prepared from 30 mice, and I-P-450$_{16\alpha}$-dependent testosterone 16α-hydroxylase activities in these microsomes were estimated as the activity inhibited by specific inhibitory antibody raised against the purified I-P-450$_{16\alpha}$ preparations as described previously (Devore et al., 1985; Noshiro et al., 1986). The activities are plotted as the function of radioactivities recovered from hybridization to the corresponding individual mouse, which was obtained in Figure 2. The squares drawn by dotted line represent three groups of offspring that exhibit low, intermediate, and high levels of I-P-450$_{16\alpha}$, respectively.

shown in histograms of Figure 2. The estimated I-P-450$_{16\alpha}$ mRNA levels in the castrated F2 offspring were separated into three groups: the low levels similar to those seen in castrated 129/J, the high levels similar to those of castrated BALB/cJ, and the intermediate levels similar to those seen in castrated 129C/F1 mice. The distribution of offspring containing the low (7), the high (10), and the intermediate (13) levels of I-P-450$_{16\alpha}$ mRNA did not differ significantly from an expected 1:2:1 ratio for additive inheritance. A linear correlation was observed between the amounts of mRNA hybridized to pf26 probe and I-P-450$_{16\alpha}$-dependent testosterone 16α-hydroxylase activity in F2 offspring as demonstrated in Figure 3.

Derepression of I-P-450$_{16\alpha}$ in Recombinant Inbred Mice. Amounts of hybridizable mRNA for I-P-450$_{16\alpha}$ in eight sham-operated and castrated 9×A recombinant inbred mice were estimated by dot hybridization and compared with those in the corresponding female mice (Figure 4). As consistent with the findings in the preceding paper (Noshiro et al., 1988), female mice of 9×AD, 9×AE, and 9×AK showed considerably higher levels of the hybridization, whereas females of the other five strains (9×AB, 9×AF, 9×AI, 9×AJ, and 9×AM) exhibited at least 20 times less hybridization. The first three strains carried genotype of Rip^h/Rip^h, and the genotype of the last five strains was Rip^l/Rip^l as previously described (Noshiro

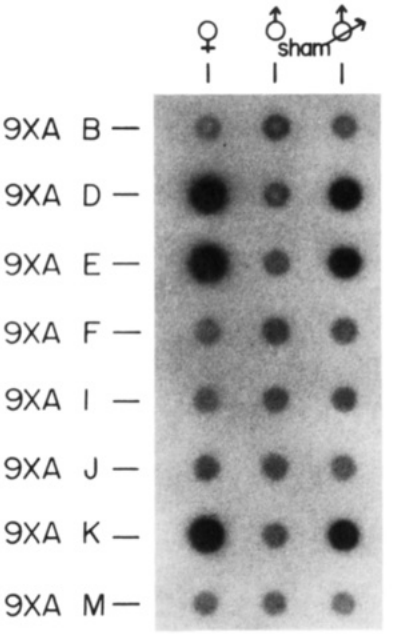

FIGURE 4: Dot hybridization of pf26 cDNA with liver poly(A+) RNA from female, sham-operated, and castrated male mice of eight recombinant inbred strains (9×A). At least three mice were killed from each strain, and livers were pooled and minced. The minced livers were separated into two equal portions, and poly(A+) RNA and microsomes were prepared from both portions. A 1-μg aliquot of poly(A+) RNA was dotted on nitrocellulose paper and hybridized with ^{32}P-labeled pf26 as described under Experimental Procedures. The hybridized paper was washed and exposed to X-ray films for 5 h.

& Negishi, 1986a). The hybridization of the pf26 cDNA probe with poly(A+) RNA from all of the sham-operated male recombinant mice was as low as that seen in the females with Rip^l/Rip^l genotypes. Castration separated eight 9×A recombinant inbred strains into two groups in terms of the amounts of pf26-hybridizable mRNA. The first group (9×AD, 9×AE, and 9×AM) showed the high levels of mRNA such as those in the corresponding female mice, and the second group (9×AB, 9×AF, 9×AI, 9×AJ, and 9×AM) demonstrated the low levels of mRNA such as those in the corresponding female mice. The results provided additional proof that the castration-dependent derepression of I-P-450$_{16\alpha}$ in male mice is regulated by a single locus and that a locus regulating this derepression in castrated mice is closely linked to the Rip locus on mouse chromosome 7.

Effect of Castration in Other Inbred Strains. Five additional inbred strains of mice (A/HeJ, C57BL/6J, C3H/HeJ, DBA/2J, and Little) were castrated and tested for I-P-450$_{16\alpha}$ mRNA levels. With the exception of Little mice, castration increased the hybridizable mRNA amounts in all of the four mouse strains to levels as high as those seen in castrated BALB/cJ mice (Figure 5). The I-P-450$_{16\alpha}$ mRNA was already high in sham-operated male Little mice, which is due to the growth hormone deficiency in the mice as previously reported (Noshiro & Negishi, 1986b). Castration, therefore, had little effect on the already induced mRNA levels in Little mice.

Effect of Steroidal and Nonsteroidal Sex Hormones on Derepression of I-P-450$_{16\alpha}$ in Castrated BALB/cJ Mice. In order to determine whether the derepression is due to the loss of androgen or estrogen in castrated BALB/cJ mice, I-P-450$_{16\alpha}$ mRNA and its enzyme activity levels were measured in livers from the castrated male mice that had been treated with dihydrotestosterone, testosterone propionate, estradiol, or diethylstilbestrol. As shown in Figure 6, testosterone pro-

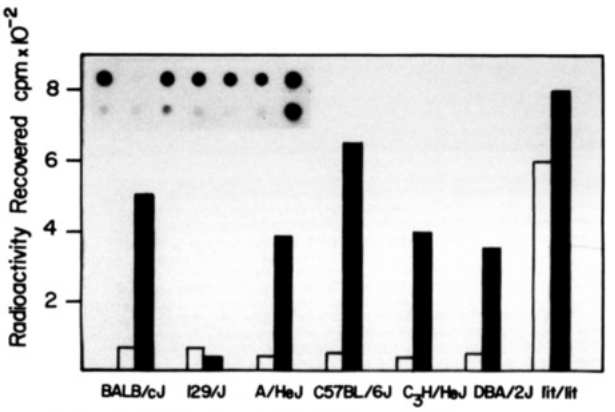

FIGURE 5: Dot hybridization of pf26 cDNA with liver poly(A+) RNA from sham-operated and castrated mice of five more inbred strains. Besides BALB/cJ and 129/J mice, five more inbred mice (A/HeJ, C57BL/6J, C3H/HeJ, DBA/2J, and lit/lit) were screened to seen the effect of castration on I-P-450$_{16\alpha}$ expression in male mice. Poly(A+) RNA (2 μg) prepared from sham-operated and castrated mice (at least four mice from each strain) was dotted on nitrocellulose paper and hybridized with the ^{32}P-labeled cDNA insert of pf26. The paper was exposed to X-ray film, and radioactivities of hybridized areas were measured by scintillation counting. The picture of dot hybridization is included here.

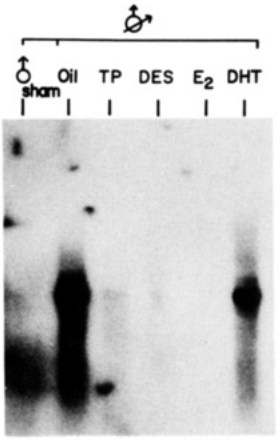

FIGURE 6: Effects of sex hormones on I-P-450$_{16\alpha}$ repression in castrated BALB/cJ mice. After 2 weeks, castrated BALB/cJ mice were treated with various hormones for 5 days. TP and DHT were dissolved in corn oil at the concentration of 1 g/mL; the concentrations of E_2 and DES in corn oil were 10 μg/mL. TP and DHT (10 mg/kg of body weight) and E_2 and DES (20 μg/kg of body weight) were injected ip once a day. Five mice from each group were killed, and pooled livers were minced. From the minced liver total poly(A+) RNA and microsomes were prepared. Poly(A+) RNAs from these mice were electrophoresed on agarose gel containing 5 mM methylmercury hydroxide, transferred to DBM paper, and then hybridized with the ^{32}P-labeled cDNA insert of pf26. The testosterone 16α-hydroxylase activities catalyzed by I-P-450$_{16\alpha}$ in microsomes from sham-operated male mice and oil-, TP-, DES-, E_2-, or DHT-treated castrated mice were determined by specific inhibitory antibody: 0.02, 0.48, <0.01, 0.03, 0.04, and 0.42 nmol min^{-1} (mg of protein)$^{-1}$, respectively. As the control, the same experiments were performed with sham-operated mice and oil-treated castrated mice and included in this figure.

pionate, as well as estradiol and diethylstilbestrol, repressed the mRNA in castrated mice, while dihydrotestosterone was the least effective hormone for repression of I-P-450$_{16\alpha}$. Since testosterone but not dihydrotestosterone can be aromatized to produce estrogen, the results obtained above suggested that estrogen but not androgen is a primary repressor of this female-specifc I-P-450$_{16\alpha}$ in male mice. The dose-dependent repression by estradiol is shown in Figure 7. As low a dose as 0.5 μg/kg of body weight was sufficient to cause the complete repression of I-P-450$_{16\alpha}$ in castrated BALB/cJ mice.

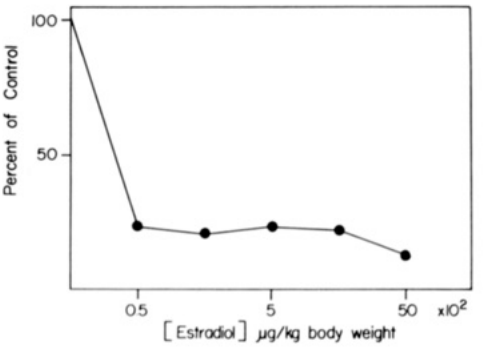

FIGURE 7: Dose dependency of repression by estradiol in castrated BALB/cJ male mice. Various amounts of estradiol (0.5, 1.0, 590, 10, and 50 μg/kg of body weight) were injected ip once a day for 5 consecutive days into castrated BALB/cJ mice. At least five castrated mice were used for each group, and corn oil was injected into the other five castrated mice as the controls. The castrated mice were housed for 2 weeks prior to hormone treatments. Poly(A) RNA was prepared and subjected to dot hybridization with ^{32}P-labeled pf26 cDNA as described under Experimental Procedures. The radioactivites recovered from dot hybridization were averaged for each dose, and the figure was constructed by taking the value of radioactivity from control mice as 100%.

DISCUSSION

Devore et al. (1985) have purified the form of P-450 (I-P-450$_{16\alpha}$) from phenobarbital-treated 129/J female mice by using testosterone 16α-hydroxylase activity as the basis for selection of fractions from columns. Subsequently, Noshiro et al. (1986) used a specific inhibitory antibody elicited against I-P-450$_{16\alpha}$ for measuring constitutive levels of I-P-450$_{16\alpha}$-dependent 16α-hydroxylase activity in liver microsomes from male and females of 129/J and BALB/cJ mice. The cross-hybridization of phenobarbital-inducible rat P-450e cDNA (R17) was also employed to estimate I-P-450$_{16\alpha}$ mRNA in liver poly(A+) RNA from these mice. The results from these experiments indicated that the constitutive expression of I-P-450$_{16\alpha}$ is a female characteristic in all inbred mice tested, except for 129/J female mice, which contain severely repressed levels of I-P-450$_{16\alpha}$ (Noshiro et al., 1986). By taking advantage of this strain difference in I-P-450$_{16\alpha}$ levels (Noshiro et al., 1988), we demonstrated that the specific expression of I-P-450$_{16\alpha}$ in female mice is regulated by a single locus named Rip on chromosome 7 in 129/J and BALB/cJ pairs.

In the present studies, we have used the differences in I-P-450$_{16\alpha}$ between 129/J and BALB/cJ pairs to investigate the genetic basis for regulation of repression of I-P-450$_{16\alpha}$ in males by sex hormones. Castration of adult males resulted in the derepression of I-P-450$_{16\alpha}$ in BALB/cJ but not in 129/J mice at the mRNA and activity levels. The patterns of derepression of I-P-450$_{16\alpha}$ in the offspring clearly indicated that this derepression in castrated male BALB/cJ mice is inherited as an autosomal additive trait and the derepression is regulated by a single locus. It should be noted that the derepression in castrated mice means the repression of I-P-450$_{16\alpha}$ in normal male mice. The depression was demonstrated in all of the tested inbred strains possessing the same Rip genotype of BALB/cJ (Rip^h/Rip^h), which included A/HeJ, C57BL/6J, C3H/HeJ, DBA/2J, 9×AD, 9×AE, and 9×AM mice. On the other hand, castration did not result in the derepression of I-P-450$_{16\alpha}$ in the mice with the Rip^l/Rip^l genotype, represented by 129/J, 9×AB, 9×AF, 9×AI, 9×AJ, and 9×AM, indicating that the locus regulating the repression of I-P-450$_{16\alpha}$ in livers of male mice is closely linked to the Rip locus on chromosome 7. We propose Ripr as the name of this locus, with r representing repression. Since an inbred mouse strain that exhibits genetic characteristics of recombination between

the two loci was not found, the possibility that Ripr and Rip are the same locus cannot be ruled out at the present time. The different inheritances of Rip(recessive) and Ripr(additive) in 129/J and BALB/cJ pairs, however, lead us to favor the hypothesis that Rip and Ripr are two different loci. On the basis of the nature of additive inheritance, Ripr is most likely to be a cis-acting genetic element.

Recently, Squires and Negishi (1988) reported that another female-specific testosterone 15α-hydroxylase (P-450$_{15\alpha}$) is derepressed not only in castrated BALB/cJ and but also in 129/J mice. Therefore, it seems that the derepression of female-specific isozymes by castration is a general mechanism in adult mice. However, since the derepression of P-450$_{15\alpha}$ by castration occurred in 129/J as well as in BALB/cJ male mice, regulation by the Ripr locus does not include P-450$_{15\alpha}$.

The repression of I-P-450$_{16\alpha}$ by testosterone and estradiol, but not by dihydrotestosterone, in the castrated mice indicated that estrogen is a repressor of this isozyme. Since we have previously reported that episodic secretion of growth hormone represses I-P-450$_{16\alpha}$ in mouse liver (Noshiro & Negishi, 1986b), estrogen is the second repressor of this P-450 so far identified. The presence of estrogen and its receptors in liver (Gordon et al., 1975; Smuk & Schwers, 1977; Frost et al., 1980) leads us to speculate on the receptor-mediated mechanism of the repression. The question then should be raised as to how the two repressors, growth hormone and estrogen, work cooperatively to repress I-P-450$_{16\alpha}$ in the liver of male mice. One possible clue is the fact that the expression of estrogen receptor in both male and female rat liver is regulated by growth hormone (Lucier et al., 1981) but not gonadol hormones (Dickson & Eisenfeld, 1979), suggesting that growth hormone is required for regulation of the estrogen receptor and estrogen is a direct repressor of I-P-450$_{16\alpha}$. At the present time, however, non-receptor-mediated mechanisms should also be considered in the future study of the repression.

The repression by estrogen of I-P-450$_{16\alpha}$ is paradoxical in that this P-450 is expressed in the liver of female mice, as the estrogen receptors are present in the liver of both males and females (Aten et al., 1978; Dickson & Eisenfeld, 1979; Siegel et al., 1985). However, further investigations such as isolation and characterization of the I-P-450$_{16\alpha}$ gene and demonstration of estrogen-dependent repression of the gene in mammalian cells or in transgenic mice well may provide a solution to the paradox.

In summary, we have demonstrated in this report that female-specific isozyme (I-P-450$_{16\alpha}$) of testosterone 16α-hydroxylase is derepressed in castrated BALB/cJ male mice but not in 129/J male mice. The derepression of I-P-450$_{16\alpha}$ was an autosomal additive trait in the BALB/cJ and 129/J pair and was regulated by a single locus named Ripr, which is located near the Rip locus on mouse chromosome 7. Estrogen was identified as a direct repressor of I-P-450$_{16\alpha}$ in the liver of male mice. This system will provide a useful model to gain insight into the genetic and hormonal regulation of sex-specific expression and repression of I-P-450$_{16\alpha}$ in mouse liver.

ACKNOWLEDGMENTS

We thank Debbie Garner for typing the manuscript.

Registry No. Testosterone 16α-hydroxylase, 37364-16-2.

REFERENCES

Aten, R. F., Dickson, R. B., & Eisenfeld, A. J. (1978) *Endocrinology (Baltimore) 103*, 1629–1635.

Aviv, A., & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1408–1412.

Bailey, J. M., & Davidson, N. (1976) *Anal. Biochem. 70*, 75–85.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Bronson, F. H., & Vom Saal, R. S. (1979) *Endocrinology (Baltimore) 104*, 1247–1255.

Colby, H. D. (1980) *Adv. Sex Horm. Res. 4*, 27–71.

Cox, R. A. (1968) *Methods Enzymol. 12B*, 120–129.

Devore, K., Harada, N., & Negishi, M. (1985) *Biochemistry 24*, 5632–5637.

Dickson, R. B., & Eisenfeld, A. J. (1979) *Biol. Reprod. 21*, 1105–1114.

Ford, H. C., Lee, E., & Engle, C. C. (1979) *Endocrinology (Baltimore) 104*, 857–861.

Frost, P. G., Reed, M. J., & James, V. H. T. (1980) *J. Steroid Biochem. 13*, 1427–1431.

Gordon, G. C., Olivo, J., Rafii, F., & Southern, A. C. (1975) *J. Clin. Endocrinol. Metab. 40*, 443–449.

Harada, N., & Negishi, M. (1984a) *J. Biol. Chem. 259*, 1265–1271.

Harada, N., & Negishi, M. (1984b) *J. Biol. Chem. 259*, 12285–12290.

Harada, N., & Negishi, M. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 2024–2028.

Lucier, G. W., Slaughter, S. R., Thompson, C., Lamartiniere, C. A., & Powell-Jones, W. (1981) *Biochem. Biophys. Res. Commun. 85*, 167–173.

Korach, K. S. (1979) *Endocrinology (Baltimore) 104*, 1324–1332.

Norsedt, G. (1984) *Mol. Cell. Endocrinol. 36*, 195–200.

Norstedt, G., & Palmiter, R. (1984) *Cell (Cambridge, Mass.) 36*, 805–812.

Noshiro, M., & Negishi, M. (1986a) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 45*, 2174.

Noshiro, M., & Negishi, M. (1986b) *J. Biol. Chem. 261*, 15923–15927.

Noshiro, M., Serabjit-Singh, C. J., Bend, J. R., & Negishi, M. (1986) *Arch. Biochem. Biophys., 244*, 857–864.

Noshiro, M., Lakso, M., Kawajiri, K., & Negishi, M. (1988) *Biochemistry* (preceding paper in this issue).

Siegel, L. I., & Fox, T. O. (1985) *Life Sci. 37*, 2151–2156.

Smuk, M., & Schwers, J. (1977) *J. Clin. Endocrinol. Metab. 45*, 1009–1012.

Squires, J., & Negishi, M. (1988) *J. Biol. Chem. 263*, 4166–4171.

Young, R. A., & Davis, R. W. (1982) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1194–1198.