0888-8809/06/$15.00/0
Printed in U.S.A.

Molecular Endocrinology 20(3):647–660

doi: 10.1210/me.2005-0328

Codependence of Growth Hormone-Responsive, Sexually Dimorphic Hepatic Gene Expression on Signal Transducer and Activator of Transcription 5b and Hepatic Nuclear Factor 4α

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Targeted disruption of the signal transducer and activator of transcription 5b gene (*STAT5b*) leads to decreased expression in male mouse liver of a male-predominant cytochrome (Cyp) 2d protein, whereas female-predominant Cyp2b proteins are increased. Presently, we characterize the effects of STAT5b deficiency on 15 specific, individual *Cyp* RNAs and other sexually dimorphic liver gene products. All seven male-specific RNAs investigated were decreased to normal female levels in STAT5b-deficient male liver, whereas five of eight female-specific RNAs, designated class I female genes, were increased in expression up to 200-fold or more. STAT5b deficiency had a much more modest effect on the expression of these genes in females. Hypophysectomy and GH replacement studies demonstrated positive GH pulse regulation of all seven male RNAs and negative GH pulse regulation of class I, but not class II, female RNAs in wild-type, but not in STAT5b-deficient, male mice. A majority of the sex-specific genes responded in parallel to the loss of STAT5b and the loss of hepatocyte nuclear factor 4α, indicating that both transcription factors are essential and suggesting they may coregulate sexually dimorphic liver gene expression. Continuous GH treatment of intact male mice, which overrides the endogenous male, pulsatile plasma GH pattern, down-regulated all seven male RNAs and induced expression of the five class I female RNAs within 4–7 d; however, induction of class II female RNAs was delayed until d 7–14. Given the slow responses of all 15 genes to changes in plasma GH status, GH regulation of sex-specific *Cyp* expression is proposed to be indirect and mediated by STAT5b- and hepatocyte nuclear factor 4α-dependent factors that may include repressors of female-specific *Cyps* and other targets of GH action. (*Molecular Endocrinology* 20: 647–660, 2006)

GH IS A PITUITARY-derived polypeptide hormone that regulates the tissue-specific expression of numerous genes involved in growth and metabolism. Pituitary GH secretion is sexually differentiated in rodents and other species, including humans, resulting in sexually dimorphic plasma GH profiles (1, 2). In the adult male rat, plasma GH levels peak at about 200 ng/ml approximately every 3.5 h, with virtually no plasma GH detectable between pulses. In female rats, GH secretion is more frequent, resulting in the near-continuous presence of GH in circulation at concentrations of 20–30 ng/ml or higher. These sexually dimorphic plasma GH patterns dictate the sexually dimorphic expression of a large number of hepatic enzymes, including cytochromes P450 (Cyps) and other enzymes involved in steroid and foreign compound metabolism (3–5). Mice also exhibit sexually dimorphic plasma GH profiles, with male mice having a longer interval between GH pulses than females (6). Hypophysectomy (hypox) and GH pulse replacement studies have established that the interpulse intervals of no or low plasma GH seen in males are required for the male-specific pattern of liver gene expression (7).

GH binds to its dimerized cell surface receptor and induces a conformational change associated with activation of the receptor-associated tyrosine kinase Janus kinase 2 (JAK2) (8). JAK2 phosphorylates key tyrosine residues in the GH receptor cytoplasmic domain, creating docking sites for downstream cytoplasmic signaling proteins, including signal transducer and activator of transcription 5b (STAT5b), one of seven mammalian STAT proteins. STAT5b, in turn, becomes phosphorylated on tyrosine 699, which enables it to dimerize and translocate to the nucleus, where it binds specific DNA response elements and activates gene transcription (9, 10). Investigation of the temporal pattern of STAT5b tyrosine phosphorylation in rat liver led to the discovery that STAT5b is directly

First Published Online October 20, 2005

Abbreviations: Cyp, Cytochrome P450; HNF4α, hepatocyte nuclear factor 4α; hypox, hypophysectomy; Mup, major urinary protein; Gst, glutathione-*S*-transferase; qPCR, quantitative real-time PCR; Slp, sex-limited protein; STAT5b, signal transducer and activator of transcription 5b.

***Molecular Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

and repeatedly activated by each incoming plasma GH pulse in males, whereas in females, nuclear STAT5b activity is generally low (11–13). A corresponding male-specific pattern of STAT5b activation has been described in mouse liver (14). STAT5b thus corresponds to a GH pulse-activated transcription factor and was proposed to mediate the stimulatory effects of male plasma GH pulses on male-specific liver *Cyp* expression (11).

This hypothesis is given strong support by the phenotype of mice with a targeted disruption of the *STAT5b* gene (15, 16). The loss of STAT5b results in GH pulse insensitivity associated with the loss of male-characteristic body growth rates (17). Western blot analysis of livers from STAT5b-deficient male mice revealed two male-predominant liver proteins, from the Cyp2d and the major urinary protein (Mup) families, that decrease in abundance, as well as female-specific Cyp2b-immunoreactive proteins that are increased, indicating a loss of sex-specific liver gene expression (15, 17). However, the specific, individual *Cyp* and *Mup* genes that are regulated by STAT5b have not been identified, hampering further mechanistic studies of this regulatory pathway. The mouse *Cyp2d* subfamily is comprised of at least six genes with 73–93% nucleotide identity, and the *Cyp2b* subfamily contains at least five genes with 55–92% nucleotide identity (18–21). *Mup* genes are also part of a multigene family containing at least seven members showing up to 97% identity (22–24).

Although STAT5b may be required for the expression of sex-dependent *Cyps* and *Mups*, STAT5b does not induce sexually dimorphic hepatic gene expression when it is activated precociously by pulsatile GH injections given to prepubertal rats (12). Moreover, STAT5b binding sites localized to the promoters of several male-specific liver *Cyp* genes confer weak transcriptional responses in GH-stimulated cells (25, 26). Thus, STAT5b may not be sufficient to induce an adult male pattern of liver *Cyp* gene expression in the absence of additional liver transcription factors. Factors reported to interact with and/or modulate STAT5 transcriptional activity include nuclear factor Y (27), glucocorticoid receptor (28, 29), peroxisome proliferator-activated receptor (30), yin-yang 1 (31), and nuclear factor I (32). Hepatocyte-enriched nuclear factors (HNFs) may also contribute to STAT5b-regulated liver gene expression. Several HNFs are regulated by GH (4, 33) and contribute to liver *Cyp* expression (34), including GH-regulated liver *Cyp* expression (25, 35–38).

HNF4α contributes to the sex specificity of liver gene expression through positive regulation of a subset of male-specific *Cyp* genes and through negative regulation of certain female-specific *Cyp*s in male mouse liver (39), as was shown using a liver-specific HNF4α-deficient mouse model (40). Presently, we use this model as well as a STAT5b-deficient mouse model (15) to investigate the GH-regulated expression of 15 sex-specific hepatic *Cyps* and other genes. Our findings reveal that many of these genes are codependent on STAT5b and HNF4α for sex-dependent expression. These findings together with our observation that sexually dimorphic *Cyp* RNAs respond slowly to the feminizing effects of continuous GH infusion lead us to propose that STAT5b and HNF4α act in concert, and by an indirect mechanism, to mediate the GH-dependent expression of *Cyps* and other sexually dimorphic liver genes.

RESULTS

STAT5b Dependence of Male-Specific Genes

To associate the effects of STAT5b deficiency with specific *Cyp* gene family members, we designed quantitative, real-time PCR (qPCR) primers that distinguish between the closely related genes that respectively comprise mouse *Cyp* gene subfamilies *2b*, *2d*, and *3a*. Primers that distinguish between *Mup* genes were also prepared. The levels of the male-specific genes *Cyp2d9* (41) and *Mup3* (also known as *Mup15*) (23, 24) were quantified by SYBR Green-based qPCR. *Mup* genes 1, 2, 6, and 8 exhibit up to 97% nucleotide identity; their expressions were therefore quantified as a group using primers that distinguish them from other genes in the *Mup* superfamily. Male-specific expression was confirmed for *Cyp2d9*, *Mup3*, and *Mup1/2/6/8*, with male/female expression ratios of 8.5, 3.6, and 7.1, respectively. Moreover, *STAT5b* gene disruption substantially reduced the expression of all three genes in both males and females (Fig. 1, A–C).

Next, we investigated whether a similar dependence on STAT5b characterizes four other mouse genes that exhibit male-specific expression in liver, namely, *Cyp7b1* (42), *Cyp4a12* (43), glutathione-*S*-transferase pi (*Gstπ*) (44), and sex-limited protein (*Slp*) (45). Male/female expression ratios were 7.9 (*Cyp7b1*), 2.9 (*Cyp4a12),* 10.3 (*Gstπ*), and 74 (*Slp*) (Fig. 1, D–G). All four genes were down-regulated in STAT5b-deficient male mice compared with the corresponding STAT5b wild-type controls. In the case of *Slp*, the loss of expression was essentially complete (Fig. 1G). The expression of *Cyp7b1* was also decreased in STAT5b-deficient females (Fig. 1D) as were the expressions of *Cyp2d9, Mup3*, and *Mup1/2/6/8* (Fig. 1, A–C). STAT5b thus plays a positive regulatory role for these four male-specific genes in both males and females (class II male genes; Table 1). In contrast, *Cyp4a12, Gstπ*, and *Slp* were substantially down-regulated in STAT5b-deficient male liver, but were unaffected by STAT5b deficiency in female liver (Fig. 1, E–G). Thus, the positive regulation of these latter genes by STAT5b is exclusively associated with males (class I male genes; Table 1).

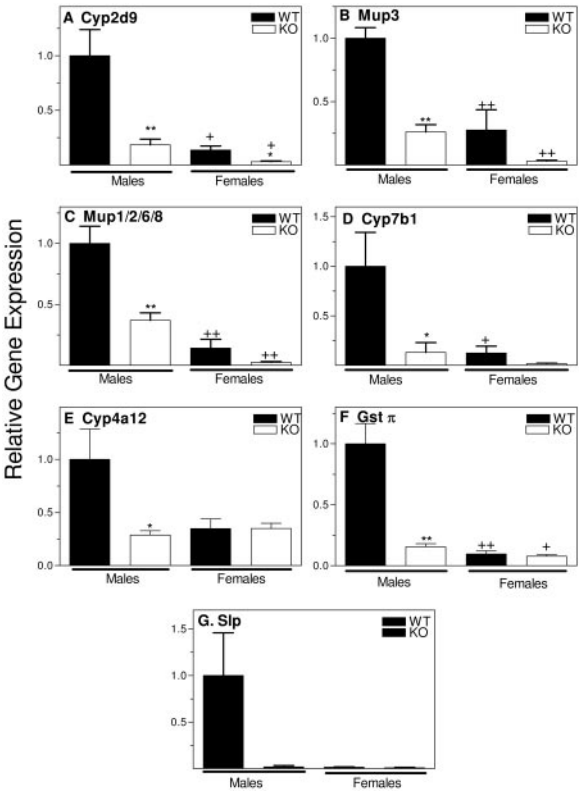

Fig. 1. Male-Specific Liver Gene Expression Is Dependent on STAT5b

Wild-type (WT) and STAT5b-deficient (KO) mouse livers were analyzed for the expression of the seven indicated male-specific genes by qPCR as described in *Materials and Methods*. Data are graphed as fold activation values (mean ± SE), normalized to the 18S rRNA content of each sample, for six (wild-type males and STAT5b-deficient males), five (wild-type females), and seven (STAT5b-deficient females) individual livers per group. Mean wild-type male RNA levels were set at 1. Statistical analyses were carried out by *t* test: ++ and +, $P < 0.01$ and $P < 0.05$, respectively, for wild-type male *vs.* wild-type female or for STAT5b-deficient male *vs.* STAT5b-deficient female; ** and *, $P < 0.01$ and $P < 0.05$, respectively, for wild-type male *vs.* STAT5b-deficient male or for wild-type female *vs.* STAT5b-deficient female.

Impact of *STAT5b* Gene Disruption on Female-Specific Genes

Next, we investigated the effects of *STAT5b* gene disruption on six female-predominant members of the *Cyp2b* and *Cyp3a* gene subfamilies, namely, *Cyp2b9, Cyp2b10* (46), *Cyp2b13* (19), *Cyp3a16* (47), *Cyp3a41*, and *Cyp3a44* (48). qPCR analysis confirmed that all six genes were much more highly expressed in female than in male mouse liver, with female/male expression ratios ranging from 33–50 for *Cyp2b10* and *Cyp2b13* to 500-fold or more for *Cyp2b9, Cyp3a16, Cyp3a41*, and *Cyp3a44* (Fig. 2). Remarkably, disruption of *STAT5b* in male liver led to strong up-regulation of all three *Cyp2b* genes, suggesting that STAT5b actively represses the expression of these genes in male mice, either directly or indirectly (Fig. 2, A–C). Moreover, the loss of STAT5b in female liver led to decreases in the expressions of these same three genes, such that sex specificity was largely abolished in the STAT5b-knockout strain. Two other female-specific genes, *Cyp2a4* and *Cyp17a1*, were also up-regulated in male liver in the absence of STAT5b, leading to a loss of sex-specific expression (Fig. 2, D and E). In contrast, the three female-specific *Cyp3a* genes did not show substantial or consistent increases in expression in STAT5b-deficient male liver (Fig. 2, F–H), such that female-specific expression was maintained in the STAT5b knockout mice. These patterns of regulation are summarized in Table 1, where the female-specific genes are grouped into two classes, I and II, based on their responses to the loss of STAT5b in male liver.

Hypox and GH Pulse Replacement Studies

Next, we investigated the GH dependence of sex-dependent gene expression in wild-type and STAT5b-deficient male mice. Pituitary hormone ablation by hypox led to substantial decreases in the expression of all seven male-specific genes in wild-type male liver (Fig. 3, bar 3 *vs.* bar 2 in each panel). In contrast, hypox of STAT5b-deficient male mice decreased the expression of only one of the seven male-specific genes (*Mup1/2/6/8*; Fig. 3C). This lack of effect of hypox in STAT5b-deficient mice may be due to the fact that pulsatile GH-induced STAT5b signaling is already abolished in these animals. To test this hypothesis, hypox male mice were treated with GH pulses given twice daily for 1 wk using a schedule that restores body weight gain in wild-type hypox mice (17). GH pulse treatment increased the expression of all seven male-specific genes, although in two cases (*GST*π and *Slp*) the response did not reach statistical significance (Fig. 3, bar 4 *vs.* bar 3). Two of the genes were restored to intact male levels (*Mup3* and *Cyp4a12*), whereas restoration of the other genes was incomplete, indicating a requirement for additional pituitary factors for full expression. No GH pulse-dependent increases were seen in the STAT5b-deficient hypox mice (Fig. 3, bar 7 *vs.* bar 6), demonstrating that STAT5b is required for GH pulse induction of male-specific liver gene expression.

The effects of hypox and GH pulse treatment on the expression of female liver genes are presented in Fig. 4. *Cyp2b9* and *Cyp2b13* were increased 200-fold or more after hypox of wild-type males (Fig. 4, A and B, bar 3 *vs.* bar 2), similar to the increases seen in response to STAT5b deficiency in intact males (bar 5 *vs.* bar 2). No additional increases in expression were seen after hypox of STAT5b-deficient mice. Moreover, GH pulse treatment substantially repressed *Cyp2b9* and *Cyp2b13* in wild-type hypox mice, but not in STAT5b-deficient hypox mice (Fig. 4, A and B, bar 4 *vs.* bar 3, compared with bar 7 *vs.* bar 6). The four other female-specific genes examined (*Cyp17a1* and the three *Cyp3a* genes) were largely unresponsive to

Table 1. Regulation of Sex-Dependent Liver Gene Expression by STAT5b and HNF4α

	STAT5b Regulation		HNF4α Regulation		GH Regulation[a]		
	Males	Females	Males	Females	Male hypox	Male hypox + GH pulse	Male + GH continous
Male-specific (class I)							
Cyp4a12, GSTπ, Slp	↑	–	↑	–	↓	↑	↓
Male-specific (class II)							
Cyp2d9, Cyp7b1 Mup3, Mup1/2/6/8	↑	↑	↑	↑[b]	↓	↑	↓
Female-specific (class IA)							
Cyp2a4, Cyp2b9, Cyp17a1	↓	–[c]	↓	–	↑[d]	↓[d]	↑
Female-specific (class IB)							
Cyp2b10,Cyp2b13	↓	–[c]	–	↑	↑[e]	↓[e]	↑
Female-specific (class II)							
Cyp3a16, Cyp3a41, Cyp3a44	–	–	–	↑	–	–	↑[f]

Sex-specific genes are grouped into each of the indicated classes based on their dependence on STAT5b and HNF4α for expression in male and female mouse livers. In the first four columns, an *up arrow* indicates the gene is positively regulated by STAT5b or HNF4α, *i.e.* gene expression is decreased in STAT5b- or HNF4α-deficient liver. A *down arrow* indicates negative regulation, *i.e.* the gene is up-regulated (derepressed) in the absence of STAT5b or HNF4α. A *dash* indicates no substantial change in gene expression. Genes belonging to three of the five classes (male classes I and II and female class IA) exhibit common patterns of dependence on STAT5b and HNF4α in both males and females. Male class I and male class II genes differ in their dependence on STAT5b and HNF4α in female liver, whereas female class IA and female class IB genes differ in their dependence on HNF4α in both sexes. The columns at the *right* indicate whether gene expression is decreased (*down arrow*) or increased (*up arrow*) in wild-type male liver after hypox, and whether gene expression increases (*up arrow*) or decreases (*down arrow*) after GH pulse treatment of hypox male mice or after treatment of intact males with a continuous GH infusion for 7–14 d. The effect of liver HNF4α deficiency on the expression of *Slp, Cyp7b1, Mup1/2/6/8, Cyp17a1*, and *Cyp3a16* was based on this study; the effects of HNF4α knockout on the expression patterns of the remaining genes are based on Ref. 39 and are included in Table 2 for reference.

[a] GH regulation in wild-type male mice. The effects of hypox and GH pulse treatment of wild-type mice summarized here were not seen in STAT5b-deficient mice (*c.f.* Figs. 3 and 4). *Cyp2a4* and *Cyp2b10* were not evaluated for the effects of hypox and GH pulse treatment on liver gene expression (see Fig. 4 legend). Cont, Continuous GH treatment for up to 14 d (see Figs. 5 and 6).

[b] *Cyp7b1* expression was too low to ascertain its dependence on HNF4α in female liver.

[c] Down-regulation was seen for several female class IA and class IB genes in STAT5b-deficient female liver, with statistical significance in the case of *Cyp2b9* (see Fig. 2).

[d] *Cyp2b9* only. *Cyp17a1* was not affected by hypox or GH pulse treatment in male liver. The effect of hypox and GH pulse treatment was not determined for *Cyp2a4.*

[e] *Cyp2b13* only. The effect of hypox and GH pulse treatment was not determined for *Cyp2b10.*

[f] Slow increase in expression; full effects were not manifest until 14 d of GH treatment.

hypox or GH pulse treatment in either wild-type or STAT5b-deficient mice (Fig. 4, C–F). These four female genes also did not display the large increases in expression that the *Cyp2b* genes showed in STAT5b-deficient males (Fig. 4, bar 5 *vs.* bar 2), indicating they are not subject to negative regulation by GH pulse-activated STAT5b. Taken together, these studies demonstrate that STAT5b, when activated by male GH pulses, positively regulates male liver gene expression while it negatively regulates a subset of female-specific genes.

HNF4α Dependence of Sex-Specific Liver Genes

Our previous work with the liver HNF4α-deficient mouse model revealed a requirement for HNF4α for the expression of *Cyp2d9*, a male-specific gene, in both male and female mouse liver, whereas two other male-specific genes, *Gstπ* and *Cyp4a12,* require HNF4α for full expression in males, but not females (39) (also see Table 2). It is now apparent that the dependence of these three male-specific genes on HNF4α mirrors their dependence on STAT5b (Fig. 1), as summarized in Table 1. We therefore investigated whether the other STAT5b-dependent, male-specific genes characterized in this study also exhibit a codependence on HNF4α. qPCR analysis revealed that the expression of *Mup3* and *Mup1/2/6/8* was strongly dependent on HNF4α in both sexes (Table 2), just as these genes were dependent on STAT5b for full expression in both sexes (Fig. 1, B and C). Moreover, *Cyp7b1* and *Slp* were found to be highly dependent on HNF4α for expression in males (Table 2), where they were also highly dependent on STAT5b (Fig. 1, D and G). We could not determine whether the latter two genes require HNF4α for expression in female liver, because their expression in females was too low to measure. We conclude that all seven male-specific genes are codependent on STAT5b and HNF4α for their sex-specific expression (Table 1).

The expression of two female-specific genes, *Cyp2b9* and *Cyp2a4*, was previously shown to be

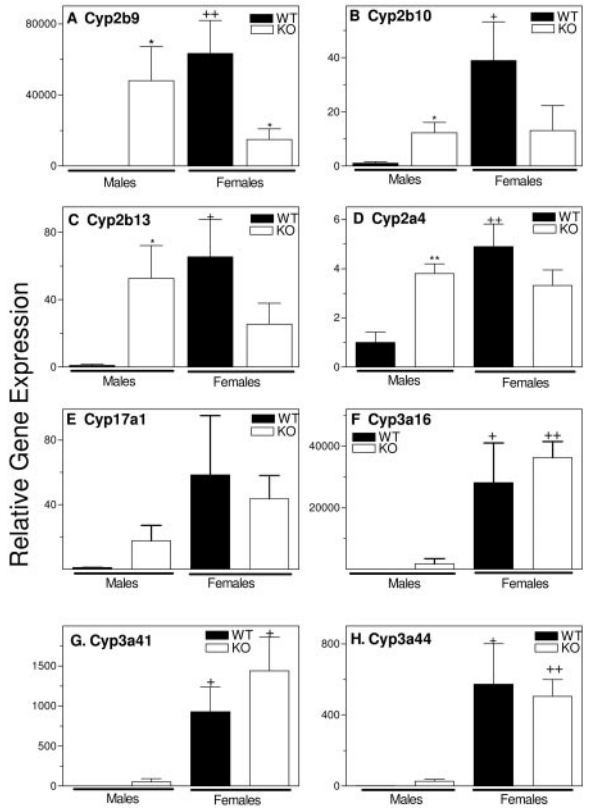

Fig. 2. Derepression of a Subset of Female-Specific Genes in STAT5b-Deficient Male Liver

Liver RNA isolated from wild-type (WT) and STAT5b-deficient (KO) mice, as described in Fig. 1, was analyzed for the expression of the eight indicated female-specific genes by qPCR as described in *Materials and Methods*. Data presentation and statistical analysis were carried out as described in Fig. 1, with the mean wild-type male RNA levels set at 1. The small increases in *Cyp3a16* and *Cyp3a41* RNAs in the STAT5b-deficient *vs.* wild-type males reflect a small increase in expression in one of the six livers analyzed. *Cyp2b9* and *Cyp3a16* were at or below the limit of detection in wild-type male liver, resulting in the exceptionally high female/male expression ratios shown on the y-axis.

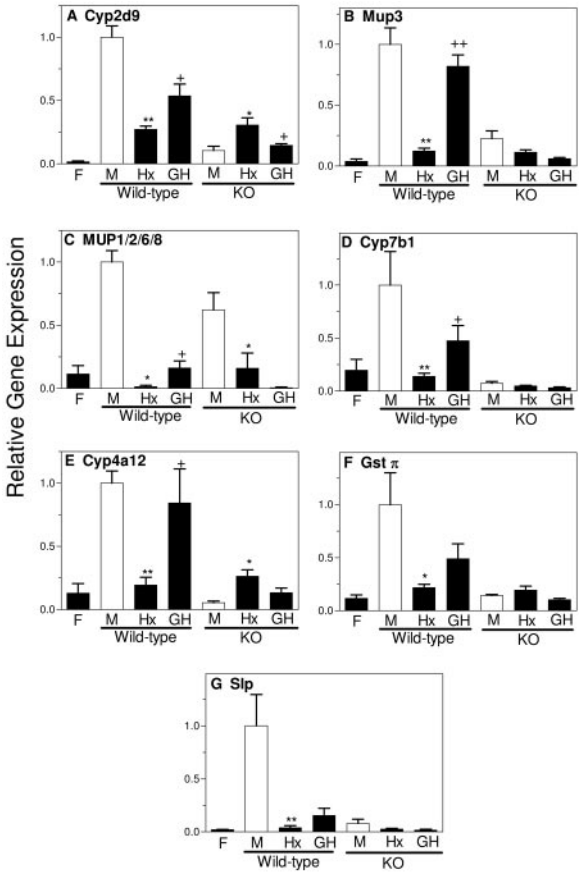

Fig. 3. Response of Male-Specific Genes to Hypox and GH Pulse Replacement

Liver RNA was isolated from livers prepared from the following groups of mice: intact wild-type females (F, bar 1; n = 3 livers) and males (M, bar 2; n = 4), hypox wild-type males without GH treatment (Hx, bar 3; n = 6), and hypox wild-type males given twice daily pulsatile GH injections for 1 wk (GH, bar 4; n = 6); intact STAT5b-deficient males (KO, bar 5; n = 4), and hypox STAT5b-deficient males without (Hx, bar 6; n = 4) or with pulsatile GH treatment (GH, bar 7; n = 5). Data shown are the mean ± SE values for each of the indicated RNAs normalized to the 18S RNA content of each sample, with the intact male (bar 2) set at a value of 1. Statistical analysis was carried out by *t* test: ** and *, $P < 0.01$ and $P < 0.05$, respectively, for hypox *vs.* intact comparisons within each strain; ++ and +, $P < 0.01$ and $P < 0.05$, respectively, for GH pulse-treated hypox *vs.* untreated hypox comparisons within each strain. Male, female, and STAT5b-deficient male groups were comprised of different liver samples from those analyzed in Figs. 1 and 2.

unaffected by the loss of HNF4α in female liver, but was up-regulated in HNF4α-deficient male liver (39) (also see Table 2). These same *Cyps* were correspondingly up-regulated in STAT5b-deficient male liver (Fig. 2). We therefore investigated whether this regulatory pattern, namely, up-regulation in males (but not females) in the absence of either STAT5b or HNF4α, is a characteristic of other female-specific genes. *Cyp2b10* and *Cyp2b13* were up-regulated in STAT5b-deficient males (Fig. 2, B and C); however, as shown previously (39), they were not substantially up-regulated in HNF4α-deficient males. Moreover, the expression of these *Cyp2b* genes was abolished in HNF4α-deficient females (39). The three female-specific *Cyp3a* genes also did not exhibit the regulatory pattern of *Cyp2b9* and *Cyp2a4*, insofar as their expression was likewise abolished in HNF4α-deficient female liver. By contrast, the loss of HNF4α expression in male, but not female, liver was associated with strong up-regulation of the female-specific *Cyp17a1* (Table 2). Thus, STAT5b and HNF4α both exert negative regulation on three of the eight female-specific genes (*Cyp2a4*, *Cyp2b9*, and *Cyp17a1*) exclusively in males (Table 1; class IA female-specific genes). By contrast, HNF4α confers a unique positive regulation in female liver on female-specific genes belonging to class IB

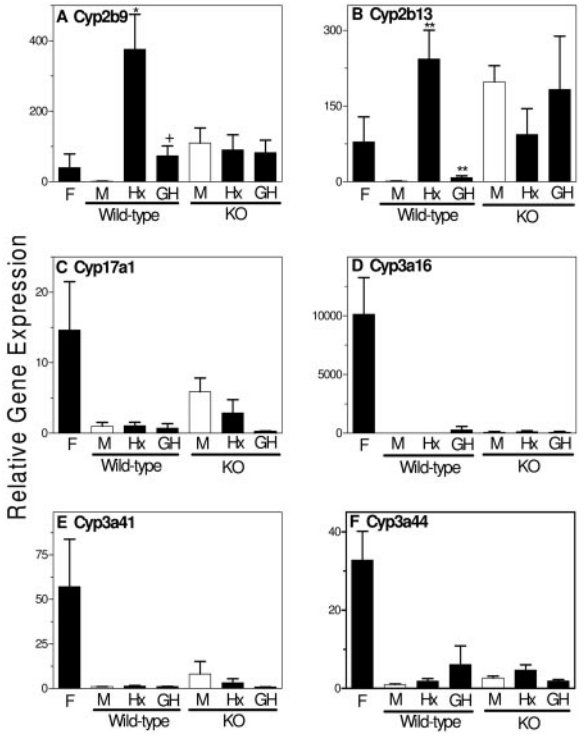

Fig. 4. Effects of Hypox and GH Pulse Treatment on Female-Specific Gene Expression

Liver RNA samples, described in Fig. 3, were analyzed for the six indicated female RNAs. Data presentation and statistical analyses were carried out as detailed in Fig. 3. The intact male liver sample (bar 2) was set at 1. *Cyp2b10* and *Cyp2a4* did not exhibit significant sex differences in expression in this set of livers and were therefore excluded. The lack of sex-specific *Cyp2b10* expression may reflect the 129/J contribution to the mixed strain used for these studies (65). The y-axis differences compared with Fig. 2 are due to differences in the basal level of female gene expression seen in males for the individual wild-type livers analyzed in this figure compared with Fig. 2.

and class II (*Cyp3a16*, *Cyp3a41*, *Cyp3a44*, *Cyp2b10*, and *Cyp2b13*).

Time Course for Feminization of Liver Gene Expression by Continuous GH Infusion

GH treatment given as a continuous infusion via an Alzet osmotic minipump overrides the male plasma GH profile and, in the rat model, feminizes liver gene expression (49–51). This feminization is associated with the suppression of liver nuclear STAT5b activity (11). The impact of continuous GH on sexually dimorphic mouse liver gene expression was assayed as a function of time of GH treatment. Continuous GH suppressed the expression of all seven male-specific genes, with suppression to near female liver levels generally achieved within 7 d (Fig. 5). In the case of *Cyp7b1*, GH-induced suppression was complete by 2 d, whereas full suppression of the other male-specific genes required at least 4 d. *Mup3* showed a decrease in expression by 4 d, followed by an apparent increase in expression after 14 d of GH treatment; however, this increase was largely due to the lack of response in two of the six individual livers examined. Apparent half-lives for decay of the male-specific RNAs ranged from 7 h (*Cyp7b1*) to 49 h (*Slp*) (Fig. 5), suggesting that differences in the time course of GH suppression may in part reflect differences in the intrinsic stability of each RNA. However, the onset of suppression was apparently delayed for at least 10 h for all male genes analyzed, except *Slp* (Fig. 5G). This observation taken together with the finding that continuous GH treatment of cultured liver cells for 2–3 h decreases activated (tyrosine-phosphorylated) STAT5b to approximately 10–20% of its peak GH pulse-induced level (52) indicate that the observed decreases are an indirect response to the loss of the normal male, pulsatile plasma GH profile.

Continuous GH treatment induced the expression of all eight female-specific genes; however, the increases in expression were unexpectedly slow, requiring up to 7–14 d for full effect (Fig. 6). Four genes, namely, the three *Cyp2b* genes and *Cyp2a4*, were near-maximally induced by d 7 of GH treatment (Fig. 6, A–D). These same *Cyp2b* genes were also subject to negative regulation by GH pulse-activated STAT5b (Fig. 4, A and B), suggesting that the feminization of these genes by continuous GH (Fig. 6) is due to the loss of this negative regulation. *Cyp17a1* responded by d 2–4, but subsequently declined (Fig. 6E). *Cyp2b13* did not respond to continuous GH infusion until d 7, and its RNA never reached untreated female liver levels, even after 14 d of treatment (Fig. 6C). An even slower response characterized the genes that were not subject to negative regulation by STAT5b or GH pulses, *i.e.* the three *Cyp3a* genes. Substantial feminization of *Cyp3a16* and *Cyp3a41* expression was not seen until d 14 (Fig. 6, F and G), when *Cyp3a16* RNA was increased to a level approximately 3.5-fold higher than the reference female control value. The delayed response of all eight female-specific genes indicates that GH induces their expression by an indirect mechanism(s). These mechanisms could include derepression of female gene expression secondary to the loss of a STAT5b-dependent, GH pulse-regulated, male-specific repressor in the case of the class I female genes, or induction of gene expression secondary to the synthesis of a female-specific activator in the case of the class II female genes (Fig. 7).

DISCUSSION

The GH-pulse activated transcription factor STAT5b is required for sex-dependent expression of several liver proteins, including male-specific Cyp2d and Mup proteins and female-specific Cyp2b proteins (15, 17). In the present study we used qPCR methods and primers that distinguish closely related mouse *Cyp* and *Mup* genes to characterize the STAT5b dependence of

Table 2. Sex-Specific Liver Gene Expression in Liver-Specific HNF4α-Deficient Male and Female Mice

Gene	HNF4α-Flox Male	HNF4α-Deficient Male	HNF4α-Flox Female	HNF4α-Deficient Female
18S rRNA	1.00 ± 0.01	0.99 ± 0.01	0.98 ± 0.04	1.01 ± 0.02
Cyp2d9	1.00 ± 0.10	0.06 ± 0.02[a]	0.09 ± 0.02[b]	≤0.01[a]
Mup3	1.00 ± 0.10	≤0.01[a]	0.21 ± 0.03[b]	≤0.01[a]
Mup1/2/6/8	1.00 ± 0.11	≤0.01[a]	0.20 ± 0.02[b]	≤0.01[a]
Cyp7b1	1.00 ± 0.20	≤0.01[a]	≤0.01[b]	0.01 ± 0.01
Cyp4a12	1.00 ± 0.16	0.43 ± 0.06[a]	0.14 ± 0.01[b]	0.17 ± 0.04
Gstπ	1.00 ± 0.18	0.45 ± 0.03[a]	0.12 ± 0.01[b]	0.31 ± 0.05[a]
Slp	1.00 ± 0.34	≤0.01[a]	≤0.01[b]	≤0.01
Cyp2b9	0.01 ± 0.01	0.93 ± 0.13[a]	1.00 ± 0.14[b]	1.13 ± 0.08
Cyp2b10	0.26 ± 0.09	0.15 ± 0.06	1.00 ± 0.19[b]	0.06 ± 0.02[a]
Cyp2b13	≤0.01	0.08 ± 0.01[a]	1.00 ± 0.14[b]	0.07 ± 0.01[a]
Cyp2a4	0.30 ± 0.06	0.79 ± 0.11[c]	1.00 ± 0.36[d]	0.95 ± 0.12
Cyp17a1	0.08 ± 0.02	0.84 ± 0.29[c]	1.00 ± 0.30[d]	0.46 ± 0.11
Cyp3a16	≤0.01	≤0.01	1.00 ± 0.39[d]	0.30 ± 0.02[c]
Cyp3a41	0.10 ± 0.00	0.09 ± 0.01	1.00 ± 0.33[b]	0.20 ± 0.07[a]
Cyp3a44	0.05 ± 0.02	0.02 ± 0.01	1.00 ± 0.50[d]	≤0.01[c]

qPCR analysis was carried out using cDNA prepared from total liver RNA isolated from HNF4α-flox male, HNF4α-deficient male, HNF4α-flox female, and HNF4α-deficient female mice (n = 8 livers/group). Data shown represent fold activation values (mean ± SE) normalized to the 18S rRNA content of each sample. Statistical differences were determined by *t* test. Data for *Cyp2d9, Cyp4α12, Gstπ, Cyp2b9, Cyp2b10, Cyp2b13, Cyp2a4*, *Cyp3a41*, and *Cyp3a44* were obtained from Ref. 39. HNF4α-Flox livers correspond to (control) livers that express normal levels of HNF4α.

[a] $P < 0.01$, HNF4α-flox *vs.* HNF4α deficient.

[b] $P < 0.01$, HNF4α-flox male *vs.* HNF4α-flox female.

[c] $P < 0.05$, HNF4α-flox *vs.* HNF4α deficient.

[d] $P < 0.05$, HNF4α-flox male *vs.* HNF4α-flox female.

15 individual sex-dependent, GH-regulated genes. Three of the male-specific genes examined were dependent on STAT5b for expression in males, but not in females (class I male-specific genes), consistent with the high nuclear STAT5b activity found in male, but not female, liver (11, 13) and with the proposal that STAT5b is an essential mediator of the effects of male plasma GH pulses on male-specific liver gene expression. By contrast, four other male-specific genes were down-regulated in the absence of STAT5b in females as well as males (class II male-specific genes). This latter finding may be explained by the presence of nuclear STAT5b activity in female liver, albeit at a much lower level than in males (13).

Five of the eight female-specific genes investigated were derepressed in STAT5b-deficient male liver, suggesting these genes may be negatively regulated by STAT5b (class IA and class IB female-specific genes). Some loss in expression was also seen in STAT5b-deficient female liver, leading to an overall loss of sex specificity in the absence of STAT5b. STAT5b may repress these female genes in male liver by a direct mechanism, or its effects may be indirect, *e.g.* mediated by a STAT5b-dependent, male-specific transcriptional repressor. However, the three class II female-specific genes, all members of the *Cyp3a* gene family, showed little or no change in expression with the loss of STAT5b in either males or females. This finding contrasts with the up-regulation of a female-specific, Cyp3a-immunoreactive protein seen previously in STAT5b-deficient males (15). This presumably corresponds to a cross-reactive, but distinctly regulated, mouse *Cyp3a* family member. The STAT5b independence of the three female-specific *Cyp3a* genes characterized in this report coupled with their lack of response to pituitary hormone ablation in males and their unusually slow induction in continuous GH-treated males (discussed below) indicate that GH regulates female-specific liver gene expression by multiple mechanisms, only some of which require STAT5b.

Hypox and GH pulse replacement studies carried out in wild-type and STAT5b-deficient mice demonstrated that the class I and class II male-specific genes are positively regulated by plasma GH pulses, and that these effects of GH require STAT5b. In the case of two of the genes, *Mup3* and *Cyp4a12*, a normal male level of liver gene expression was restored to the hypox mice by pulsatile GH replacement, in agreement with our previous observation for Mup protein in mouse urine (17), whereas only partial restoration was observed for the other male genes. The incomplete response of the latter genes may reflect the inadequacy of the twice-daily GH injection protocol to mimic the normal male mouse plasma GH profile (6), or it may indicate a requirement for additional pituitary hormones for high level male liver gene expression. Hypox of male mice led to dramatic induction (*i.e.* derepression) of two class I female genes, *Cyp2b9* and *Cyp2b13*, and GH pulse treatment substantially reversed these effects in wild-type mice, but not in STAT5b-deficient mice. By contrast, the class II female genes were not up-regulated in response to hypox, in agreement with a previous report (48) and consistent with our finding that these genes were not

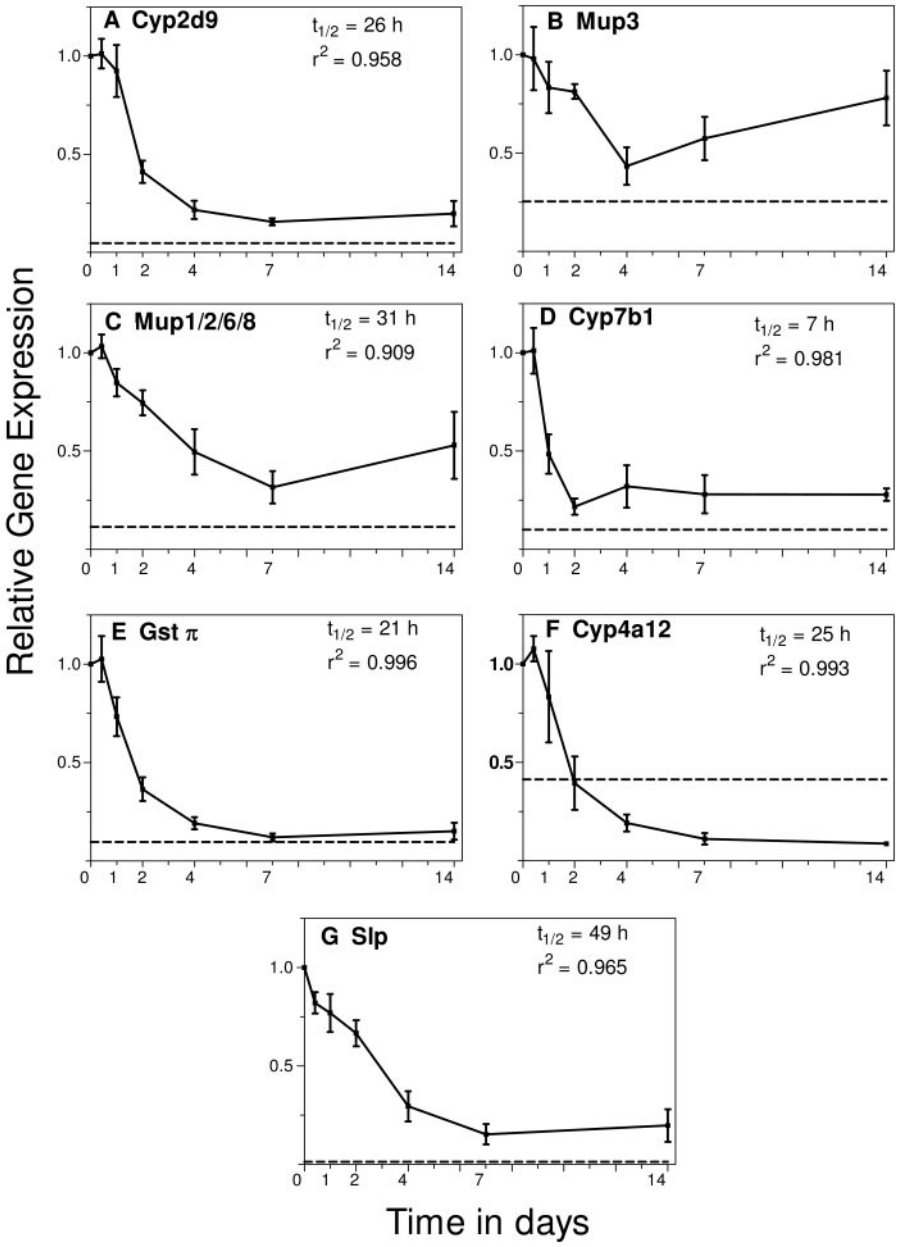

Fig. 5. Time Course for Feminization of Male Gene Expression by Continuous GH

Male mice were treated with GH as a continuous infusion over a period of 10 h to 14 d using Alzet osmotic minipumps, and livers were analyzed for expression of the indicated male-specific genes by qPCR as described in *Materials and Methods*. Data were normalized to the 18S rRNA content of each sample and are graphed relative to the average RNA level of the male sham controls (n = 14 livers), which was set at 1. The female liver RNA level of each gene is indicated by a *horizontal dashed line*. Data represent the mean ± SE for six or seven livers per group. Analysis of the rates of RNA decay after initiation of GH treatment was determined as described in *Materials and Methods*, with $t_{1/2}$ and r^2 values as indicated. The half-life value could not be determined for *Mup3*.

induced upon loss of GH pulse-induced STAT5b signaling in STAT5b-deficient mice. Thus, although the sex specificity of the female class I genes and the male genes of both classes can be attributed to the STAT5b-dependent actions of pulsatile GH, the sex specificity of the female class II genes involves a distinct mechanism, one that requires GH, but is independent of STAT5b. Conceivably, this mechanism may involve a STAT5b-independent GH signaling pathway, such as MAPK or phosphotidylinositol 3-kinase, or the participation of other sex-dependent endocrine factors, such as steroid hormones. Indeed, glucocorticoids have been shown to contribute to the GH-dependent regulation of *Cyp3a41* expression (53).

Continuous GH infusion of adult male mice feminized the overall pattern of liver gene expression, with

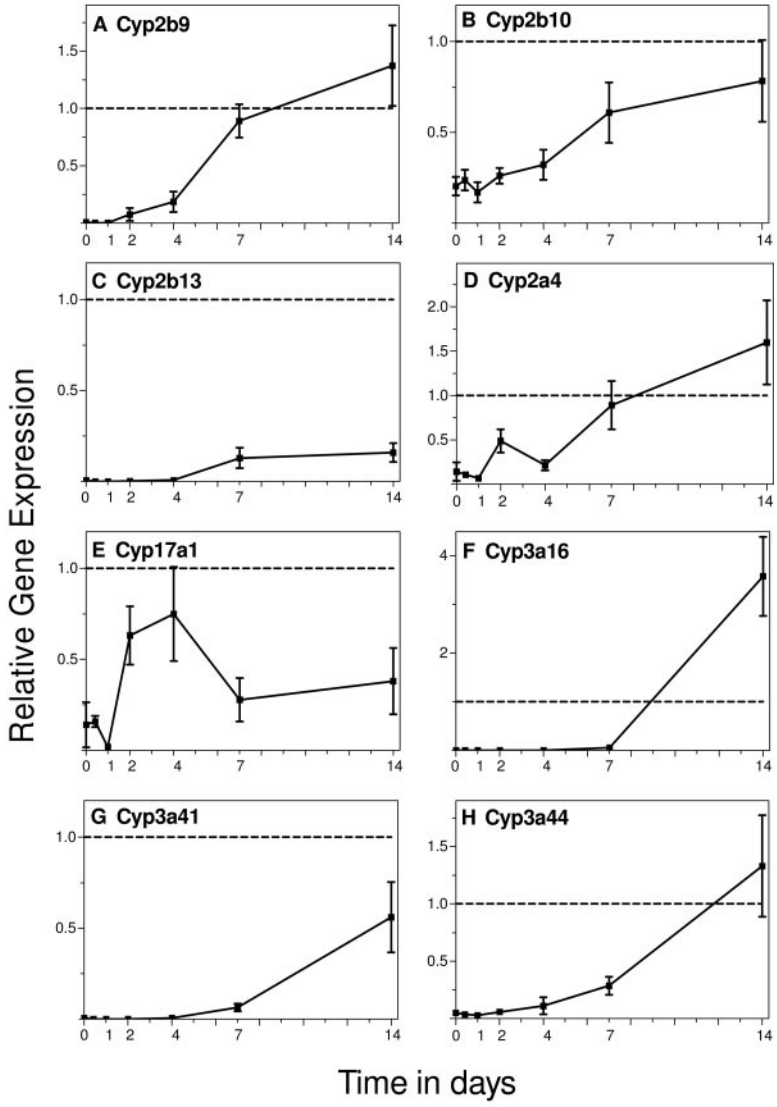

Fig. 6. Up-Regulation of Female-Specific Gene Expression by Continuous GH Treatment

Continuous GH-treated male mouse liver RNA samples, described in Fig. 5, were assayed for expression of the indicated female-specific genes by qPCR. Data are presented as in Fig. 5, except that the RNA levels were graphed relative to the mean untreated female control liver RNA level (*horizontal dashed line*; n = 5 livers), which was set at 1. Three genes showed apparent stress-dependent (but GH-independent) increases in expression at the early time points after Alzet minipump implantation: *Cyp2b10* showed GH-independent increases at 10 h, 1 d, and 2 d; *Cyp2a4* at 10 h and 1 d; and *Cyp17a1* at 10 h (data not shown). For these genes, data shown at the above-mentioned time points were adjusted for the stress response by setting the expression levels of the sham pump controls to that of the untreated male controls.

all seven male-specific RNAs down-regulated to female levels within 4–7 d. This time course could in part be a reflection of the intrinsic half-lives of these RNAs. Liver P450 RNA half-lives range from approximately 7 h (rat CYP2B) (54) to 14–19 h (rabbit CYP1A and CYP3A) (55) to 24 h or more (human CYP1A) (56), similar to the apparent RNA decay rates presently seen in GH-treated male livers. However, an apparent delay of at least 10 h characterized the down-regulation of six of the seven male RNAs that we studied. By contrast, no such delay characterized the induction of the early GH response gene suppressor of cytokine signaling 2, whose RNA is more highly expressed in female than in male mouse liver and was substantially induced after 10 h of GH treatment (our unpublished observations). Given the rapid down-regulation of nuclear STAT5b activity seen in continuous GH-treated liver cells (52) and in liver *in vivo* (11), the delay in the continuous GH down-regulation of male liver gene expression that we report points to a multistep mechanism, by which continuous GH initially down-regulates a STAT5b-induced, male-specific activator or, perhaps, up-regulates a normally female-specific repressor of the male genes, which, in turn, mediates the observed down-regulation of male-specific *Cyp* and *Mup* genes.

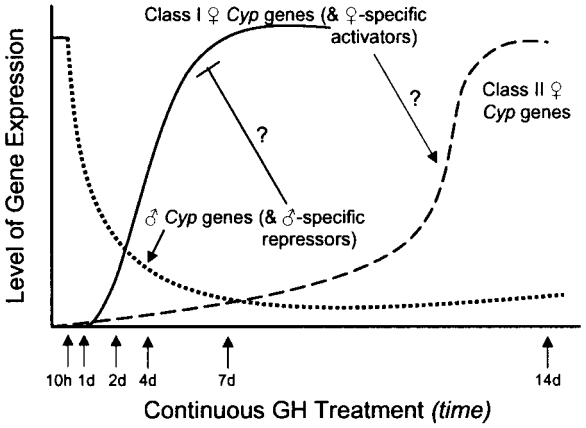

Fig. 7. Time Course for Feminization of Sex-Dependent Liver Genes by Continuous GH Infusion

Shown is a diagram of the time course for feminization of liver gene expression in male mice given a continuous infusion of GH lasting from 10 h up to 14 d, based on data shown in Figs. 5 and 6. The figure presents the responses of the major groups of sex-specific liver genes, classified on the basis of their regulation by STAT5b and HNF4α (Table 1). Strong decreases in the expression of class I and class II male genes were observed by d 4–7 of GH treatment (*dotted line*), followed by induced expression of class I female genes. Class II female genes, belonging to the *Cyp3a* subfamily, were induced by continuous GH at a later time point (*long dashes*). Also shown is the hypothetical role of repressors and activators, which are proposed to mediate the effects of STAT5b and HNF4α, on each of the classes of sex-dependent liver genes (*arrows* with *question marks*).

The induction of female gene expression in the continuous GH-treated male mice proceeded with an even more striking delay compared with the loss of male gene expression. Thus, class IA and class IB female-specific genes generally required approximately 7 d of GH treatment for maximal induction, and in the case of the class II female genes, up to 14 d were required for feminization. The observed increases in female gene expression are thus likely to be secondary events, *e.g.* subsequent to the decay of a long-lived male-specific repressor (Fig. 7). Other GH-stimulated target genes, such as suppressor of cytokine signaling genes, are activated rapidly, within 20–30 min of GH treatment (57), and in this context, the sexually dimorphic hepatic genes examined in this study may all be considered secondary response genes.

Taken together, the present findings support our earlier conclusion, based on the analysis of a limited number of Cyp- and Mup-immunoreactive proteins (15), that STAT5b is essential for the sexual dimorphism of male mouse liver and furthermore demonstrate that STAT5b has limited effects in female mouse liver, where many of the genes studied were largely unaffected by the loss of STAT5b. Although STAT5b is thus necessary, it is not sufficient to induce an adult male pattern of liver gene expression. This conclusion is supported by the finding that an adult male pattern of liver gene expression is not achieved when STAT5b is activated precociously in prepubertal rats given exogenous GH pulses (12) or when STAT5b is activated by very low-amplitude GH pulses given to hypox rats (58). These observations together with the weak transcriptional response associated with STAT5b binding to certain male-specific liver *Cyp* promoters (25, 26) indicate a requirement for additional regulatory factors. These factors are likely to include HNF4α, a liver-enriched transcription factor that plays an essential role in liver *Cyp* expression (34, 40) and can positively regulate two male-specific genes while inhibiting the expression of a subset of female-specific genes in male liver (39). Our further investigation of the role of HNF4α revealed that expression of the class II male genes requires HNF4α in both sexes, whereas expression of the class I male genes requires HNF4α in males only. Interestingly, these same regulatory patterns were seen in STAT5b-deficient mice. Moreover, the three class IA female genes showed a common dependence on STAT5b and HNF4α for negative regulation of expression in males.

These findings lead us to propose, as a working hypothesis, that HNF4α- and GH pulse-activated STAT5b work in a cooperative manner to activate male-specific genes while concomitantly repressing class IA female-specific genes in male liver. One mechanism could involve direct transcriptional stimulatory or inhibitory actions of STAT5b and HNF4α, acting in concert, on the sex-specific liver promoters. Indeed, several STAT5b and HNF4α consensus DNA binding motifs have been identified in the 5′-regulatory regions of multiple *Cyp* genes, including *Cyp2d9* and *Cyp2a4* (25, 39, 59), supporting the possibility that these factors directly regulate the sex-specific promoters. However, the apparent 10-h delay that characterized the down-regulation of six of the seven male genes by continuous GH compared with the 2–3 h required for continuous GH to suppress STAT5b activity (52), noted above, strongly suggests that the effects of GH on these male genes are indirect. Furthermore, the fact that STAT5b is well characterized as a transcriptional activator, but is not known to serve as a strong, direct-acting transcriptional repressor, supports the proposal that STAT5b indirectly suppresses female gene expression in male mouse liver.

We therefore favor the hypothesis that STAT5b and HNF4α control sex-dependent liver *Cyp* and *Mup* gene expression by an indirect mechanism. One possibility is that the loss of STAT5b in the hypothalamus interrupts the feedback inhibitory action of GH on pituitary GH release leading to feminization of plasma GH profiles, which could help explain the loss of male liver gene expression and the induced expression of female genes seen in STAT5b-deficient males. However, the apparent GH pulse-insensitivity of hypox STAT5b-deficient mouse liver (Figs. 3 and 4) suggests a more direct liver regulatory role of STAT5b. Accordingly, a second possibility is that intermediary transcriptional activators and repressors that are directly induced by GH pulse-activated STAT5b and/or HNF4α

regulate sex-specific liver *Cyp* genes. For example, STAT5b and HNF4α may positively regulate the class I male genes by inducing the expression of an immediate-early response gene that corresponds to a male-specific transcriptional activator. Precedent is provided by the finding that GH-activated STAT5b acts in cooperation with HNF4α to transcriptionally activate another liver transcription factor, *HNF6* (60). Similarly, STAT5b, acting in concert with HNF4α, could induce the expression of a liver-specific repressor that, in turn, inhibits the expression of class IA female genes. The increase in class I female RNAs closely follows the down-regulation of all seven male liver RNAs examined, suggesting that one of the down-regulated male genes could be a repressor that contributes to the observed up-regulation of the class I female genes. Similarly, the female-specific genes up-regulated in mice deficient in STAT5b or HNF4α could include transcriptional repressors that target male genes and contribute to the major decreases in expression seen for both class I and class II male-specific genes in the absence of these transcription factors.

In conclusion, our findings using STAT5b- and HNF4α-deficient mouse models lead us to propose that these two transcription factors mediate the sex-dependent effects of GH on liver gene expression primarily in an indirect manner, via their regulation of immediate-early response genes, which may include transcriptional activators and repressors that act on downstream *Cyps* and other sex-dependent, GH-regulated targets. Additional studies, including genome-wide microarray analysis, will be required to test this hypothesis and identify these putative STAT5b- and HNF4α-dependent regulators of sex-specific gene expression. The present studies thus provide important new insight into the sexual dimorphism that characterizes mouse liver gene expression. These findings may further our understanding of sexually dimorphic *CYP* expression and GH regulation in human liver, which are characteristic of CYP3A4 (61–63) and CYP2B6 (64), two CYP enzymes that play important roles in drug and steroid metabolism in humans.

MATERIALS AND METHODS

Knockout Mice

STAT5b and *HNF4α* gene-disrupted mice have been described previously (15, 40). Briefly, STAT5b-deficient mice (129 × BALB/c) were generated by insertion of a neomycin resistance cassette at the *Bam*HI site interrupting the codon for amino acid 181 (15). Liver tissues from 8- to 9-wk-old wild-type and STAT5b-deficient mice, originally obtained from Dr. Helen Davey (AgResearch, Hamilton, New Zealand), were used in this study. Hypox and GH pulse replacement by ip injection at 2 μg/g body weight/injection, twice daily for 1 wk, were carried out as described previously (17). Liver-specific HNF4α-deficient mice (129/SV × C57B6 × FVB) were generated by albumin promoter-regulated Cre-loxP-mediated deletion of exons 4 and 5 of the *HNF4α* gene (40). Livers from 48-d-old liver-specific HNF4α-deficient and HNF4α-flox (control) mice obtained from Y. Inoue and F. J. Gonzalez (National Cancer Institute, Bethesda, MD) were described previously (39). Livers were snap-frozen in liquid nitrogen at the time of collection and stored at −80 C until use.

Continuous GH Treatment

Male and female mice of the ICR strain, 8–10 wk of age, were purchased from Taconic Farms, Inc. (Germantown, NY). Mice were housed in a temperature- and humidity-controlled environment with a 12-h light, 12-h dark cycle. Untreated male and female mice (n = 5/group) were killed by cervical dislocation, their livers were collected, snap-frozen in liquid nitrogen, and stored at −80 C until use. Alzet osmotic pumps [models 1003D (3-d pump), 1007D (7-d pump), and 1002 (2-wk pump), purchased from Durect Corp., Cupertino, CA] were filled with recombinant rat GH (purchased from Dr. A. F. Parlow, National Hormone and Peptide Program, Harbor-University of California-Los Angeles Medical Center, Torrance, CA) dissolved in buffer [30 mM $NaHCO_3$ (pH 8.3) and 0.15 M NaCl containing 100 μg/ml rat albumin]. Pumps were filled with buffer alone in the case of sham-treated mice. Pumps were implanted s.c. under ketamine and xylazine anesthesia. GH was infused at a rate of 20 ng/g body weight·h (7) for periods ranging from 10 h to 14 d. Livers were collected from six or seven GH-treated male mice and from two or three mice implanted with buffer-filled pumps at each time point. Mice were killed by cervical dislocation, and livers were removed and stored at −80 C. Animal procedures were approved by the institutional animal care and use committee at Boston University.

Primer Design and qPCR Analysis

Each of the 15 sex-specific mouse liver genes chosen for analysis gave similar sex-specific expression patterns across all three wild-type mouse strains analyzed in this study, minimizing the impact of strain background differences among the various mouse models. qPCR primers specific to each gene of interest were designed using Primer Express software (Applied Biosystems, Foster City, CA). To ensure the specificity of each primer pair, sequences of closely related members of each *Cyp* and *Mup* gene family were aligned with each other, and primers were designed in the regions of greatest sequence divergence. Primers were selected to include at least two nucleotide mismatches with each related RNA. The specificity of each primer was validated using the National Center for Biotechnology BLAST program. qPCR primers selected for *Mup1/2/6/8* were unable to distinguish among *Mup* genes 1, 2, 6, and 8, because the percent nucleotide identities of these genes ranged up to 97%. Similarly, the *Gstπ* primers did not distinguish Gstπ1 and Gstπ2 RNAs (~98% identity) (44). Expression profiles obtained using these *Mup* and *Gstπ* primers therefore reflect the expression pattern of the most abundant RNAs in each group. Primer sequences are shown in Table 3 or were described previously (39). Quantification of relative levels of hepatic RNAs was achieved by real-time qPCR using SYBR Green I chemistry. Total RNA was isolated from individual mouse livers (~0.1 g frozen liver tissue) using TRIzol reagent (Invitrogen Life Technologies, Inc., Carlsbad, CA) according to the manufacturer's protocol. The isolated RNA was treated with 1 U RQ1 ribonuclease-free deoxyribonuclease (Promega Corp., Madison, WI), followed by heating for 5 min at 75 C. cDNA was synthesized by reverse transcription of 0.5 or 1 μg total RNA in 20-μl reactions containing random hexamers and murine leukemia virus reverse transcriptase (Applied Biosystems). Triplicate samples of each qPCR mixture, each containing 4 μl SYBR Green I PCR Master Mix (Applied Biosystems), were pipetted into separate wells of a 384-well

Table 3. Mouse qPCR Primer Sets and GenBank Accession Numbers

Gene	Oligo No.	GenBank No.	Amplicon (nt)	Forward Primer	Reverse Primer
Mup3	1237/1238	NM_010845	280–331	GAAGAGTGCACCGAAATGACTG	TGCCAGCCTTTTCTGTTTGTT
Mup1/2/6/8	1239/1240	—[a]	—[a]	GACTTTTTCTGGAGCAAATCCATG	GAGCACTCTTCATCTCTTACAG
Cyp3a16	1388/1389	NM_007820	362–412	CAAACCGGCAGGATTTTTTC	GAGAGATGGATTTACTCATTATCCCC
Cyp7b1	1414/1415	NM_007825	1145–1195	TGAGGTTCTGAGGCTGTGCTC	TCCTGCACTTCTCGGATGATG
Cyp17a1	1408/1409	NM_007809	232–251	TGCCCCTGGTGGGTAGTCTA	CATGCATATGACCACGTCTGG
Slp	1243/1244	NM_011413	3150–3206	GACCAAGGACCATGCTGTGC	AACTGCTGGATCCGAACGTGG

qPCR primer pairs were designed as described in *Materials and Methods.* Nucleotide (nt) numbering indicating the position of the resultant PCR amplicon is based on the indicated GenBank accession numbers. Primer sequences and GeneBank accession numbers for other genes characterized in this study can be found in Ref. 39.

[a] Accession numbers and nucleotide positions were: NM_031188 (nt 281–351) for *Mup1*, BC012259 (nt 229–307) for *Mup2*, AK013259 (nt 237–314) for *Mup6*, and XO4115 (nt 39–117) for *Mup8*.

plate and run through 40 cycles on an ABS 7900HT sequence detection system (Applied Biosystems) (39). Dissociation curves were generated after each qPCR run to ensure that a single, specific product was amplified. Data are graphed as fold activation values, normalized to the 18S rRNA content of each sample. Statistical analyses were carried out by *t* test using PRISM software version 4 (GraphPad, Inc., San Diego, CA) for pairwise comparisons indicated in each figure. $P < 0.05$ was considered significant. Apparent half-lives for decay of the male-specific liver RNAs in continuous GH-treated livers were determined using a nonlinear curve fit and a one-phase exponential decay equation (GraphPad PRISM).

Acknowledgments

This paper is dedicated to the memory of Dr. Helen W. Davey (1957–2005), who was instrumental in developing the STAT5b-deficient mouse model used in the present study.

Received August 10, 2005. Accepted October 11, 2005.

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This work was supported by National Institutes of Health Grant DK-33765 (to D.J.W.).

M.G.H., E.V.L., and D.J.W. have nothing to declare.

REFERENCES

1. Jansson JO, Eden S, Isaksson O 1985 Sexual dimorphism in the control of growth hormone secretion. Endocr Rev 6:128–150
2. Veldhuis JD, Anderson SM, Shah N, Bray M, Vick T, Gentili A, Mulligan T, Johnson ML, Weltman A, Evans WS, Iranmanesh A 2001 Neurophysiological regulation and target-tissue impact of the pulsatile mode of growth hormone secretion in the human. Growth Hormone IGF Res 11:S25–S37
3. Shapiro BH, Agrawal AK, Pampori NA 1995 Gender differences in drug metabolism regulated by growth hormone. Int J Biochem Cell Biol 27:9–20
4. Wiwi CA, Waxman DJ 2004 Role of hepatocyte nuclear factors in growth hormone-regulated, sexually dimorphic expression of liver cytochromes P450. Growth Factors 22:79–88
5. Mode A, Ahlgren R, Lahuna O, Gustafsson JA 1998 Gender differences in rat hepatic CYP2C gene expression: regulation by growth hormone. Growth Horm IGF Res 8(Suppl B):61–67
6. MacLeod JN, Pampori NA, Shapiro BH 1991 Sex differences in the ultradian pattern of plasma growth hormone concentrations in mice. J Endocrinol 131:395–399
7. Waxman DJ, Pampori NA, Ram PA, Agrawal AK, Shapiro BH 1991 Interpulse interval in circulating growth hormone patterns regulates sexually dimorphic expression of hepatic cytochrome P450. Proc Natl Acad Sci USA 88:6868–6872
8. Wan Y, McDevitt A, Shen B, Smythe ML, Waters MJ 2004 Increased site 1 affinity improves biopotency of porcine growth hormone. Evidence against diffusion dependent receptor dimerization. J Biol Chem 279:44775–44784
9. Herrington J, Carter-Su C 2001 Signaling pathways activated by the growth hormone receptor. Trends Endocrinol Metab 12:252–257
10. Darnell Jr JE 1997 STATs and gene regulation. Science 277:1630–1635
11. Waxman DJ, Ram PA, Park SH, Choi HK 1995 Intermittent plasma growth hormone triggers tyrosine phosphorylation and nuclear translocation of a liver-expressed, Stat 5-related DNA binding protein. Proposed role as an intracellular regulator of male-specific liver gene transcription. J Biol Chem 270:13262–13270
12. Choi HK, Waxman DJ 2000 Plasma growth hormone pulse activation of hepatic JAK-STAT5 signaling: developmental regulation and role in male-specific liver gene expression. Endocrinology 141:3245–3255
13. Choi HK, Waxman DJ 1999 Growth hormone, but not prolactin, maintains, low-level activation of STAT5a and STAT5b in female rat liver. Endocrinology 140: 5126–5135
14. Sueyoshi T, Yokomori N, Korach KS, Negishi M 1999 Developmental action of estrogen receptor-α feminizes the growth hormone-Stat5b pathway and expression of Cyp2a4 and Cyp2d9 genes in mouse liver. Mol Pharmacol 56:473–477
15. Udy GB, Towers RP, Snell RG, Wilkins RJ, Park S-H, Ram PA, Waxman DJ, Davey HW 1997 Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. Proc Natl Acad Sci USA 94: 7239–7244
16. Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G, Ihle JN 1998 Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. Cell 93:841–850
17. Davey HW, Park SH, Grattan DR, McLachlan MJ, Waxman DJ 1999 STAT5b-deficient mice are growth hor-

mone pulse-resistant. Role of STAT5b in sex-specific liver p450 expression. J Biol Chem 274:35331–35336
18. Wong G, Itakura T, Kawajiri K, Skow L, Negishi M 1989 Gene family of male-specific testosterone 16α-hydroxylase (C-P-450(16α)) in mice. Organization, differential regulation, and chromosome localization. J Biol Chem 264:2920–2927
19. Lakso M, Masaki R, Noshiro M, Negishi M 1991 Structures and characterization of sex-specific mouse cytochrome P-450 genes as members within a large family. Duplication boundary and evolution. Eur J Biochem 195: 477–486
20. Marc N, Damon M, Fautrel A, Guillouzo A, Corcos L 1999 Isolation of a cyp2b10-like cDNA and of a clone derived from a cyp2b10-like pseudogene. Biochem Biophys Res Commun 258:11–16
21. Keeney DS 1998 The novel skin-specific cytochrome P450 Cyp2b19 maps to proximal chromosome 7 in the mouse, near a cluster of Cyp2 family genes. Genomics 53:417–419
22. Clark AJ, Chave-Cox A, Ma X, Bishop JO 1985 Analysis of mouse major urinary protein genes: variation between the exonic sequences of group 1 genes and a comparison with an active gene out with group 1 both suggest that gene conversion has occurred between MUP genes. EMBO J 4:3167–3171
23. Held WA, Gallagher JF, Hohman CM, Kuhn NJ, Sampsell BM, Hughes Jr RG 1987 Identification and characterization of functional genes encoding the mouse major urinary proteins. Mol Cell Biol 7:3705–3712
24. Shahan K, Gilmartin M, Derman E 1987 Nucleotide sequences of liver, lachrymal, and submaxillary gland mouse major urinary protein mRNAs: mosaic structure and construction of panels of gene-specific synthetic oligonucleotide probes. Mol Cell Biol 7:1938–1946
25. Park SH, Waxman DJ 2001 Inhibitory Cross-talk between STAT5b and Liver Nuclear Factor HNF3β. Impact on the regulation of growth hormone pulse-stimulated, male-specific liver cytpchrome P-450 gene expression. J Biol Chem 276:43031–43039
26. Subramanian A, Teixeira J, Wang J, Gil G 1995 A STAT factor mediates the sexually dimorphic regulation of hepatic cytochrome P450 3A10/lithocholic acid 6β-hydroxylase gene expression by growth hormone. Mol Cell Biol 15:4672–4682
27. Subramanian A, Wang J, Gil G 1998 STAT 5 and NF-Y are involved in expression and growth hormone-mediated sexually dimorphic regulation of cytochrome P450 3A10/lithocholic acid 6β-hydroxylase. Nucleic Acids Res 26:2173–2178
28. Stoecklin E, Wissler M, Schaetzle D, Pfitzner E, Groner B 1999 Interactions in the transcriptional regulation exerted by Stat5 and by members of the steroid hormone receptor family. J Steroid Biochem Mol Biol 69:195–204
29. Wyszomierski SL, Yeh J, Rosen JM 1999 Glucocorticoid receptor/signal transducer and activator of transcription 5 (STAT5) interactions enhance STAT5 activation by prolonging STAT5 DNA binding and tyrosine phosphorylation. Mol Endocrinol 13:330–343
30. Shipley JM, Waxman DJ 2003 Down-regulation of STAT5b transcriptional activity by ligand-activated peroxisome proliferator-activated receptor (PPAR) α and PPARγ. Mol Pharmacol 64:355–364
31. Bergad PL, Towle HC, Berry SA 2000 Yin-yang 1 and glucocorticoid receptor participate in the Stat5-mediated growth hormone response of the serine protease inhibitor 2.1 gene. J Biol Chem 275:8114–8120
32. Mukhopadhyay SS, Wyszomierski SL, Gronostajski RM, Rosen JM 2001 Differential interactions of specific nuclear factor I isoforms with the glucocorticoid receptor and STAT5 in the cooperative regulation of WAP gene transcription. Mol Cell Biol 21:6859–6869
33. Rastegar M, Lemaigre FP, Rousseau GG 2000 Control of gene expression by growth hormone in liver: key role of a network of transcription factors. Mol Cell Endocrinol 164:1–4
34. Akiyama TE, Gonzalez FJ 2003 Regulation of P450 genes by liver-enriched transcription factors and nuclear receptors. Biochim Biophys Acta 1619:223–234
35. Lahuna O, Fernandez L, Karlsson H, Maiter D, Lemaigre FP, Rousseau GG, Gustafsson J, Mode A 1997 Expression of hepatocyte nuclear factor 6 in rat liver is sex-dependent and regulated by growth hormone. Proc Natl Acad Sci USA 94:12309–12313
36. Delesque-Touchard N, Park SH, Waxman DJ 2000 Synergistic action of hepatocyte nuclear factors 3 and 6 on CYP2C12 gene expression and suppression by growth hormone-activated STAT5b. Proposed model for female specific expression of CYP2C12 in adult rat liver. J Biol Chem 275:34173–34182
37. Wiwi CA, Waxman DJ 2005 Role of hepatocyte nuclear factors in transcriptional regulation of male-specific CYP2A2. J Biol Chem 280:3259–3268
38. Endo M, Takahashi Y, Sasaki Y, Saito T, Kamataki T 2005 Novel gender-related regulation of CYP2C12 gene expression in rats. Mol Endocrinol 19:1181–1190
39. Wiwi CA, Gupte M, Waxman DJ 2004 Sexually dimorphic P450 gene expression in liver-specific hepatocyte nuclear factor 4α-deficient mice. Mol Endocrinol 18:1975–1987
40. Hayhurst GP, Lee YH, Lambert G, Ward JM, Gonzalez FJ 2001 Hepatocyte nuclear factor 4α (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. Mol Cell Biol 21:1393–1403
41. Harada N, Negishi M 1984 Mouse liver testosterone 16α-hydroxylase (cytochrome P-450(16)α). Purification, regioselectivity, stereospecificity, and immunochemical characterization. J Biol Chem 259:12285–12290
42. Stapleton G, Steel M, Richardson M, Mason JO, Rose KA, Morris RG, Lathe R 1995 A novel cytochrome P450 expressed primarily in brain. J Biol Chem 270:29739–29745
43. Heng YM, Kuo CS, Jones PS, Savory R, Schulz RM, Tomlinson SR, Gray TJ, Bell DR1997 A novel murine P-450 gene, Cyp4a14, is part of a cluster of Cyp4a and Cyp4b, but not of CYP4F, genes in mouse and humans. Biochem J 325:741–749
44. Bammler TK, Smith CA, Wolf CR 1994 Isolation and characterization of two mouse Pi-class glutathione *S*-transferase genes. Biochem J 298:385–390
45. Varin-Blank N, Dondi E, Tosi M, Hernandez C, Boucontet L, Gotoh H, Shiroishi T, Moriwaki K, Meo T 1998 Male-specific transcription initiation of the C4-Slp gene in mouse liver follows activation of STAT5. Proc Natl Acad Sci USA 95:8750–8755
46. Nemoto N, Sakurai J 1995 Glucocorticoid and sex hormones as activating or modulating factors for expression of Cyp2b-9 and Cyp2b-10 in the mouse liver and hepatocytes. Arch Biochem Biophys 319:286–292
47. Itoh S, Satoh M, Abe Y, Hashimoto H, Yanagimoto T, Kamataki T 1994 A novel form of mouse cytochrome P450 3A (Cyp3a-16). Its cDNA cloning and expression in fetal liver. Eur J Biochem 226:877–882
48. Sakuma T, Endo Y, Mashino M, Kuroiwa M, Ohara A, Jarukamjorn K, Nemoto N 2002 Regulation of the expression of two female-predominant CYP3A mRNAs (CYP3A41 and CYP3A44) in mouse liver by sex and growth hormones. Arch Biochem Biophys 404:234–242
49. Mode A, Norstedt G, Simic B, Eneroth P, Gustafsson JA 1981 Continuous infusion of growth hormone feminizes hepatic steroid metabolism in the rat. Endocrinology 108: 2103–2108
50. Ahluwalia A, Clodfelter KH, Waxman DJ 2004 Sexual dimorphism of rat liver gene expression: regulatory role of growth hormone revealed by deoxyribonucleic acid microarray analysis. Mol Endocrinol 18:747–760

51. Laz EV, Wiwi CA, Waxman DJ 2004 Sexual dimorphism of rat liver nuclear proteins: regulatory role of growth hormone. Mol Cell Proteomics 3:1170–1180
52. Gebert CA, Park SH, Waxman DJ 1999 Down-regulation of liver JAK2-STAT5b signaling by the female plasma pattern of continuous growth hormone stimulation. Mol Endocrinol 13:213–227
53. Sakuma T, Kitajima K, Nishiyama M, Endo Y, Miyauchi K, Jarukamjorn K, Nemoto N 2004 Collaborated regulation of female-specific murine Cyp3a41 gene expression by growth and glucocorticoid hormones. Biochem Biophys Res Commun 314:495–500
54. De Waziers I, Garlatti M, Bouguet J, Beaune PH, Barouki R 1995 Insulin down-regulates cytochrome P450 2B and 2E expression at the post-transcriptional level in the rat hepatoma cell line. Mol Pharmacol 47:474–479
55. Daujat M, Clair P, Astier C, Fabre I, Pineau T, Yerle M, Gellin J, Maurel P 1991 Induction, regulation and messenger half-life of cytochromes P450 IA1, IA2 and IIIA6 in primary cultures of rabbit hepatocytes. CYP 1A1, 1A2 and 3A6 chromosome location in the rabbit and evidence that post-transcriptional control of gene IA2 does not involve mRNA stabilization. Eur J Biochem 200:501–510
56. Lekas P, Tin KL, Lee C, Prokipcak RD 2000 The human cytochrome P450 1A1 mRNA is rapidly degraded in HepG2 cells. Arch Biochem Biophys 384:311–318
57. Larsen L, Ropke C 2002 Suppressors of cytokine signalling: SOCS. Review article. Acta Pathol Microbiol Immunol Scand 110:833–844
58. Verma AS, Dhir RN, Shapiro BH 2005 Inadequacy of the Janus kinase 2/signal transducer and activator of transcription signal transduction pathway to mediate episodic growth hormone-dependent regulation of hepatic CYP2C11. Mol Pharmacol 67:891–901
59. Yokomori N, Nishio K, Aida K, Negishi M 1997 Transcriptional regulation by HNF-4 of the steroid 15[α]-hydroxylase P450 (Cyp2a-4) gene in mouse liver. J Steroid Biochem Mol Biol 62:307–314
60. Lahuna O, Rastegar M, Maiter D, Thissen JP, Lemaigre FP, Rousseau GG 2000 Involvement of STAT5 (signal transducer and activator of transcription 5) and HNF-4 (hepatocyte nuclear factor 4) in the transcriptional control of the hnf6 gene by growth hormone. Mol Endocrinol 14:285–294
61. Wolbold R, Klein K, Burk O, Nussler AK, Neuhaus P, Eichelbaum M, Schwab M, Zanger UM 2003 Sex is a major determinant of CYP3A4 expression in human liver. Hepatology 38:978–988
62. Jaffe CA, Turgeon DK, Lown K, Demott-Friberg R, Watkins PB 2002 Growth hormone secretion pattern is an independent regulator of growth hormone actions in humans. Am J Physiol 283:E1008–E1015
63. Yu AM, Fukamachi K, Krausz KW, Cheung C, Gonzalez FJ 2005 Potential role for human cytochrome P450 3A4 in estradiol homeostasis. Endocrinology 146:2911–2919
64. Lamba V, Lamba J, Yasuda K, Strom S, Davila J, Hancock ML, Fackenthal JD, Rogan PK, Ring B, Wrighton SA, Schuetz EG 2003 Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J Pharmacol Exp Ther 307:906–922
65. Noshiro M, Serabjit-Singh CJ, Bend JR, Negishi M 1986 Female-predominant expression of testosterone 16α-hydroxylase ("I"-P-450(16)α) and its repression in strain 129/J Arch Biochem Biophys 244:857–864

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