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CENTENNIAL PERSPECTIVE

Sex Differences in the Expression of Hepatic Drug Metabolizing Enzymes

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ABSTRACT

Sex differences in pharmacokinetics and pharmacodynamics characterize many drugs and contribute to individual differences in drug efficacy and toxicity. Sex-based differences in drug metabolism are the primary cause of sex-dependent pharmacokinetics and reflect underlying sex differences in the expression of hepatic enzymes active in the metabolism of drugs, steroids, fatty acids and environmental chemicals, including cytochromes P450 (P450s), sulfotransferases, glutathione transferases, and UDP-glucuronosyltransferases. Studies in the rat and mouse liver models have identified more than 1000 genes whose expression is sex-dependent; together, these genes impart substantial sexual dimorphism to liver metabolic function and pathophysiology. Sex differences in drug metabolism and pharmacokinetics also occur in humans and are due in part to the female-predominant expression of CYP3A4, the most important P450 catalyst of drug metabolism in human liver. The sexually dimorphic expression of P450s and other liver-expressed genes is regulated by the temporal pattern of plasma growth hormone (GH) release by the pituitary gland, which shows significant sex differences. These differences are most pronounced in rats and mice, where plasma GH profiles are highly pulsatile (intermittent) in male animals versus more frequent (nearly continuous) in female animals. This review discusses key features of the cell signaling and molecular regulatory mechanisms by which these sex-dependent plasma GH patterns impart sex specificity to the liver. Moreover, the essential role proposed for the GH-activated transcription factor signal transducer and activator of transcription (STAT) 5b, and for hepatic nuclear factor (HNF) 4α, as mediators of the sex-dependent effects of GH on the liver, is evaluated. Together, these studies of the cellular, molecular, and gene regulatory mechanisms that underlie sex-based differences in liver gene expression have provided novel insights into the physiological regulation of both xenobiotic and endobiotic metabolism.

Individual differences in drug metabolism and pharmacokinetics contribute to the individual-to-individual variation that characterizes the responses to many drugs and other foreign chemicals and presents a major challenge in clinical pharmacology. Individual variation in the expression of major drug-metabolizing enzymes (DMEs), including cytochromes P450 (P450s), sulfotransferases, glutathione transferases, and UDP-glucuronosyltransferase, is associated with substantial individual differences in the bioavailability and clearance of drugs and other xenobiotics. Given the crucial role of hepatic DMEs in regulating the pharmacological and biological activity of drugs as well as steroid and other endobiotics, it is important to understand the regulatory features that lead to individual differences in the expression of DMEs. Factors that contribute to interindividual differences in DME expression and drug metabolism include genetic polymorphisms (Hines et al., 2008), prior or concomitant exposure to drugs and environmental chemicals (Urquhart et al., 2007), dietary factors (Moon et al., 2006; Murray, 2007), pregnancy (Anderson, 2005a), diseased states (Mann, 2006), epigenetic factors (Szyf, 2007), and endogenous hormonal factors, which change with age and differ between male and female subjects (Cotreau et al., 2005; Kennedy, 2008).

Genetic polymorphisms of DMEs influence the clinical outcome of an estimated 20 to 25% of all drug therapies (In-

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ABBREVIATIONS: DME, drug-metabolizing enzyme; P450, cytochrome P450; GH, growth hormone; IGF, insulin-like growth factor; STAT, signal transducer and activator of transcription; JAK, Janus kinase; HNF, hepatic nuclear factor.

gelman-Sundberg, 2004; Eichelbaum et al., 2006). Several key drug-metabolizing human P450 genes are highly polymorphic, with more than 350 distinct alleles identified for the 57 known human P450 genes. A well studied example is *CYP2D6*, where genetic polymorphisms contribute to large interindividual variability in the metabolism of many antidepressants, antipsychotics, analgesics, and anticancer drugs (Ingelman-Sundberg et al., 2007). Interindividual differences also arise from the induction of P450s and other DMEs after exposure to lipophilic drugs and environmental chemicals. For example, phenobarbital and other drugs can increase the rates of metabolism of many drugs and chemicals by increasing the expression of individual P450 genes (Waxman and Azaroff, 1992). This P450 induction response is mediated by ligand-activated transcription factors (nuclear receptors) that function as sensors of foreign compounds and can activate a large number of DME genes in liver and other tissues (Waxman, 1999; Handschin and Meyer, 2003; Timsit and Negishi, 2007), with the associated potential for drug interactions (Jana and Paliwal, 2007; Sinz et al., 2008). The xenobiotic-activated nuclear receptors constitutive androstane receptor, pregnane X-receptor, and peroxisome proliferator-activated receptor-α respond to a wide range of xenochemicals and induce the expression of *CYP2B*, *CYP3A*, and *CYP4A* genes, respectively. Aryl hydrocarbon receptor is a receptor/transcription factor that induces *CYP1* family members and certain other DME genes upon binding polycyclic aromatic hydrocarbons and other drugs and environmental chemicals (Ramadoss et al., 2005). Hepatic expression of DMEs can also be altered by pathophysiological conditions such as diabetes, inflammation, alcohol consumption, and protein-calorie malnutrition (Kim and Novak, 2007; Morgan et al., 2008). In addition, circadian factors regulate the expression of certain DMEs (Gachon et al., 2006) and contribute to the dosing time-dependence of drug activity and toxicity (Levi and Schibler, 2007).

Sex-based differences[1] in pharmacokinetics and pharmacodynamics are well recognized (Fletcher et al., 1994; Gandhi et al., 2004; Franconi et al., 2007) and can be an important source of individual differences in drug responses. Sex-based differences in pharmacokinetics reflect differences in bioavailability, distribution, metabolism, and/or excretion. Sex hormones influence bioavailability through effects on gastrointestinal motility; estrogen inhibits gastric emptying. Sex differences in pharmacokinetics can result from sex differences in distribution, which can be caused by differences in body weight (lower in women), body fat (higher in women), plasma volume (lower in women, but varies through the menstrual cycle and during pregnancy), and organ blood flow (higher in women). In addition, body-weight effects on glomerular filtration rate can contribute to lower renal clearance in women in the case of drugs that are actively eliminated via the kidney (Gandhi et al., 2004). However, sex differences in metabolism are thought to be the primary determinant of sex differences in pharmacokinetics. Male-female differences in the metabolism of barbiturates were documented in rats as early as 1932 (Nicholas and Barron, 1932) and were later found to characterize many drugs and steroids, as shown in both rat and mouse models (Kato, 1974; Skett, 1988). These sex differences in metabolism are now known to reflect sex differences in the expression of specific, individual hepatic P450 enzymes and other DMEs (Shapiro et al., 1995). Sex-linked differences in P450-dependent drug and steroid metabolism are also found in hamster (Teixeira and Gil, 1991; Sakuma et al., 1994), dog (Lin et al., 1996; Hay Kraus et al., 2000), chicken (Pampori and Shapiro, 1993), and fish (Gray et al., 1991; Lee et al., 1998). Sex differences in human hepatic P450-catalyzed drug metabolism are well documented, but are less dramatic than in the rat (Tanaka, 1999; Parkinson et al., 2004; Scandlyn et al., 2008). This review discusses some of the pharmacological consequences of the observed sex differences in drug metabolism. The role of hormones, in particular GH, in establishing and maintaining these sex differences is discussed, and recent mouse and rat model studies that elucidate the underlying cellular signaling and molecular regulatory mechanisms by which pituitary GH secretory profiles dictate sex-based differences in DME expression in the liver are evaluated, focusing on factors that regulate the transcription of genes that code for P450 enzymes.

Sex Differences in Hepatic Drug Metabolism

Sex-based differences characterize the metabolism of many drugs commonly used in humans (Gandhi et al., 2004; Anderson, 2005b; Schwartz, 2007); 6 to 7% of new drug applications that include a sex analysis show at least 40% difference in pharmacokinetics between men and women (Anderson, 2005b). For example, acetaminophen clearance rates are 22% higher in men than in women because of a higher rate of glucuronidation (Miners et al., 1983). The higher bioavailability of aspirin in women than in men has been linked to decreased conjugation with glycine and glucuronic acid (Franconi et al., 2007). Men show a higher rate of clearance of the benzodiazepines diazepam, chlordiazepoxide, and olanzapine (MacLeod et al., 1979; Roberts et al., 1979; Bigos et al., 2008). Likewise, women display greater sensitivity to diazepam, which can lead to impairment of psychomotor skills (Palva, 1985). CYP3A4, the predominant cytochrome P450 catalyst of oxidative metabolism in human liver, is expressed at a higher protein and mRNA level in women than in men (Hunt et al., 1992; Wolbold et al., 2003; Diczfalusy et al., 2008). Accordingly, certain CYP3A4 drug substrates, including cyclosporine (Kahan et al., 1986), erythromycin (Austin et al., 1980), and nifedipine (Krecic-Shepard et al., 2000), show higher clearance rates in women. CYP3A4-catalyzed hepatic microsomal *N*-dechloroethylation of the anticancer drug ifosfamide is also more rapid in women, suggesting that women could be more susceptible to the neurotoxic side affects associated with this metabolic pathway (Schmidt et al., 2001). CYP3A4 also metabolizes steroids, such as cortisol, whose conversion to 6β-hydroxycortisol is more rapid in women than in men and serves as a biomarker for CYP3A4 metabolic activity (Inagaki et al., 2002).

Higher CYP2B6 activity has been observed in women than in men of Hispanic origin (Lamba et al., 2003). There is also evidence for higher CYP2A6 activity in women (Sinues et al.,

[1] Although the term "gender differences" is often used in the medical literature to describe male-female differences, "sex" is the preferred term when describing biologically determined physiological, or pathophysiological, differences between male and female members of a species. "Gender" is a social construct that refers to an individual's self-representation as masculine or feminine, which, unlike "sex," can be influenced by cultural factors (Gray, 2007).

2008), although that could, in part, reflect the use of estrogen-containing contraceptives, given the estrogen responsiveness of the human *CYP2A6* gene (Higashi et al., 2007). CYP1A2 substrates, including caffeine and the antipsychotic agents olanzapine and clozapine, show higher clearance rates in men (Schwartz, 2007). As a result, women show greater improvement in psychotic symptoms but suffer a greater number of adverse side effects after treatment with these drugs. Men also show more rapid clearance of CYP2E1 substrates (e.g., chlorzoxazone) and certain CYP2D6 substrates, including propanolol, metoprolol, dextromethorphan, desipramine, and mirtazapine (Franconi et al., 2007; Schwartz, 2007). Increased rates of glucuronidation (UGT2B15 activity with oxazepam as substrate) also characterize male compared with female livers (Court et al., 2004). Overall, women are more likely to experience adverse drug reactions compared with men, due in part to sex differences in metabolic activity (Rademaker, 2001), with notable effects for cancer chemotherapeutic drugs (Mader, 2006; Wang and Huang, 2007) and antiretroviral agents (Ofotokun, 2005).

Studies carried out in rats and mice have established that sex-based differences in liver function also characterize many phase II DMEs, including sulfotransferases (Klaassen et al., 1998; Clodfelter et al., 2006; Kocarek et al., 2008), glutathione transferases (Srivastava and Waxman, 1993; Clodfelter et al., 2006; Knight et al., 2007), and UDP-glucuronosyltransferases (Takeuchi et al., 2004; Clodfelter et al., 2006; Buckley and Klaassen, 2007). In addition to drugs and other xenobiotics, P450s and other DMEs metabolize endogenous sex steroids (Waxman, 1988; Song and Melner, 2000; You, 2004; Chouinard et al., 2008), suggesting that the sex differentiation of drug metabolism reflects a need for sex-specific steroid metabolism. The sexual dimorphism of liver gene expression is not confined to DMEs; it is quite extensive and affects more than 1000 individual genes in both rats and mice (Clodfelter et al., 2006, 2007; Yang et al., 2006; Wauthier and Waxman, 2008). These include plasma lipoproteins (Oscarsson et al., 1991; Rudling et al., 1992), pheromone binding proteins (Johnson et al., 1995), regulators of fatty acid homeostasis (Amador-Noguez et al., 2005; Cheung et al., 2007), nuclear receptors (such as peroxisome proliferator-activated receptor-α and prolactin receptor) (Robertson et al., 1990; Jalouli et al., 2003), and other transcription factors, including sterol regulatory element binding protein (Lahuna et al., 1997; Améen et al., 2004; Laz et al., 2007). The extent to which sex differences occur in human liver is largely unknown; however, sex differences in genes such as these are likely to contribute to the observed sex differences in hepatic pathophysiology (Yokoyama et al., 2005), including differences in susceptibility to hepatocellular carcinoma seen both in animal models and in the clinic (Rogers et al., 2007; Shimizu et al., 2007).

Gonadal and Pituitary Hormonal Determinants of Hepatic Sex Differences

Early studies carried out in rats demonstrated that sex-based differences in P450 activities were abolished by castration, were re-established by testosterone replacement (Kato and Onoda, 1970), and were reversed by treatment with estradiol (Kramer et al., 1978). It was initially presumed that the gonadal hormones exerted their effects on steroid and drug metabolism via direct actions on the liver. However, an intact pituitary gland was later found to be required for testosterone and estradiol to regulate hepatic corticosterone metabolism, a sexually dimorphic metabolic activity (Colby et al., 1973). Several other groups later confirmed the requirement of an intact pituitary gland for gonadal hormones to regulate sex-dependent drug metabolism (Denef, 1974; Gustafsson and Stenberg, 1974; Lax et al., 1974). Further investigations implicated GH, a 191-amino acid protein hormone secreted by the anterior pituitary gland, in mediating the effects of gonadal hormones on liver drug and steroid metabolism. Wilson first observed that GH treatment of male but not female rats suppressed total P450 protein and drug metabolic activity (Wilson, 1973; Wilson and Bass, 1973). These effects of GH were later shown by Kramer et al. (1978) and Rumbaugh and Colby (1980) to mimic those of estradiol and were manifested in testosterone-treated female rats, establishing a clear link between sex steroids and the responsiveness of hepatic drug metabolism to GH treatment.

A key finding was the subsequent discovery by Edén (1979) that GH was secreted by the pituitary gland in a sexually dimorphic manner: in adult male rats, GH release into the bloodstream occurs in discrete pulses every ~3.5 h, with little or no GH detectable between pulses. In adult female rats, however, GH secretion is more frequent, resulting in a more continuous presence of GH in circulation, albeit at a much lower level than peak male levels (Tannenbaum and Martin, 1976; Edén, 1979). Mice also display sex differences in the ultradian pattern of GH secretion, male mice having less frequent GH pulses and longer baseline intervals compared with female mice (MacLeod et al., 1991). These sex-dependent patterns of pituitary GH release are set ("imprinted") by the action of gonadal hormones on the hypothalamus during the neonatal period, are manifested at puberty, and continue into adulthood (Jansson et al., 1985). The sexually dimorphic expression of liver steroid metabolic activity was later shown to be determined by the temporal pattern of GH stimulation: continuous but not pulsatile GH treatment restored female liver enzyme profiles in hypophysectomized female rats, whereas estrogen treatment of intact male rats feminized liver enzyme profiles in association with a feminization of the plasma GH profile (Mode et al., 1982). Subsequent studies by many laboratories demonstrated that 1) the differential effect of pulsatile versus continuous GH administration on hepatic drug and steroid metabolism (mimicking male- and female-like plasma GH patterns, respectively) is due to the action of GH on individual P450s and other sex-specific liver genes, and 2) these regulatory responses are largely determined at the level of gene transcription (Mode and Gustafsson, 2006; Waxman and O'Connor, 2006).

Humans also display plasma GH patterns that are sex-dependent and gonadal hormone-regulated (Veldhuis, 1998; Veldhuis and Bowers, 2003), with sex-dependent effects on certain human DMEs, albeit much smaller than those seen in rats and mice. CYP3A4 drug metabolic activity is increased in acromegalic patients and in men treated with GH-releasing hormone every 2 h (Watkins et al., 1993). In other studies, GH treatment of elderly men using a once-daily treatment schedule led to induction of CYP1A2 and, to a lesser extent, inhibition of CYP2C19 metabolic activity, but no effects were seen on CYP2D6 and CYP3A4 activity (Jürgens et al., 2002). However, in a separate study that investigated the

pattern-dependence of GH responses in GH-deficient male and female patients, pulsatile GH was found to be more effective than continuous GH treatment with respect to stimulation of osteocalcin (a GH-responsive marker of bone formation) and down-regulation of CYP1A2 metabolic activity, whereas several other GH targets (serum IGF-1, serum IGF binding protein-3, and hepatic CYP3A4 activity) were increased more effectively using a continuous GH treatment schedule (Jaffe et al., 2002). Thus, pulsatile and continuous GH treatment can induce distinct responses of individual GH target genes in humans, in a manner similar to that in the rat and mouse models.

GH regulation of CYP3A4 can be recapitulated in primary human hepatocyte cultures, where CYP3A4 protein and mRNA can be induced by continuous GH treatment and suppressed by intermittent (pulsatile) GH (Liddle et al., 1998; Dhir et al., 2006). Moreover, as discussed elsewhere (Waxman and O'Connor, 2006), in transgenic mouse lines that contain the complete *CYP3A4* gene, including 5′ and 3′ flanking DNA and the associated *cis* regulatory sequences, hepatic CYP3A4 expression shows a pattern of postnatal developmental regulation and adult sex specificity indistinguishable from that of several endogenous female-specific mouse *Cyp3a* gene products. Thus, hepatic CYP3A4 protein and RNA are expressed in immature male transgenic mice but are strongly down-regulated and become undetectable by 6 weeks of age, whereas in female mice, the *CYP3A4* transgene is expressed in both immature and adult livers. Furthermore, continuous GH treatment of the transgenic male mice substantially increased liver CYP3A4 protein and RNA (Cheung et al., 2006). Thus, the human *CYP3A4* gene contains all of the DNA sequence elements required to respond to the endogenous mouse hormonal environment, leading to a pattern of postnatal developmental regulation, adult sexual dimorphism, and plasma GH-responsiveness very similar to that of the endogenous female-specific, GH-regulated mouse *Cyp3a* genes. Sex-specificity and GH regulation have also been observed for *CYP2C18* and *CYP2C19* in a transgenic mouse liver model, where introduction of these tandem human genes together with flanking regulatory sequences results in hepatic expression that is male-specific and suppressed by continuous GH treatment (Löfgren et al., 2008). Further investigation will be required to ascertain the relevance of these findings in transgenic mice to the expression of these P450 genes in humans, which differ from mice in terms of their plasma GH profiles and other physiological factors.

Sex differences in DME expression have been observed in the kidney, where testosterone rather than GH is the proximal stimulus of male-specific gene expression. For example, the male-specific expression of rat *CYP4A2* is stimulated by GH pulses in the liver but by testosterone in the kidney (Sundseth and Waxman, 1992). This, together with similar findings in studies of other DMEs in a mouse model (Henderson and Wolf, 1991), indicates that, in the kidney, male-specific DMEs are regulated by androgen receptor-dependent mechanisms, rather than by GH-dependent mechanisms. It is conceivable that a direct androgen-stimulatory mechanism might be ineffective in hepatocytes, where androgen receptor levels are generally low and high steroid metabolic activity may lead to rapid and extensive inactivation of androgens.

Class I and Class II Sex-Specific Genes

Male-specific genes can be classified based on their response to pituitary hormone ablation. Class I male-specific genes require pituitary hormones (principally GH) for full expression and are therefore decreased in expression after hypophysectomy, whereas class II male-specific genes are primarily regulated by the repressive actions of the female GH pattern in both rats (Table 1) (Wauthier and Waxman, 2008) and mice (Table 2; V. Wauthier and D. J. Waxman, unpublished observations). Thus, plasma GH pulses positively regulate class I male-specific P450 genes, whereas class II male-specific P450 genes do not require GH; indeed, they are expressed in liver at their highest levels when GH is ablated by hypophysectomy (Table 1). Rat *CYP2C11* is a prototypic class I male-specific gene: it is induced after 4 weeks of age by the male, pulsatile GH secretion pattern, whereas a more continuous, female-like plasma GH profile abolishes *CYP2C11* expression (Fig. 1 and Table 1). A GH-free interpulse interval of at least 100 to 140 min is required for full expression of *CYP2C11* in male rat liver (Waxman et al., 1991; Agrawal and Shapiro, 2001), whereas GH ablation in male rats decreases *CYP2C11* expression to 25 to 30% of intact male levels (Morgan et al., 1985; Janeczko et al., 1990). Class II male-specific genes include rat genes *CYP2A2*, *CYP2C13*, and *CYP3A2* (Yamazoe et al., 1986; Waxman et al., 1988; McClellan-Green et al., 1989). These liver-expressed genes are not down-regulated by hypophysectomy; i.e., they do not require male GH pulse stimulation. Most strikingly, class II male-specific genes are up-regulated to near normal adult male liver levels in hypophysectomized female rats (Yamazoe et al., 1986; Waxman et al., 1988; McClellan-Green et al., 1989), evidencing the strong suppressive action of the female pituitary hormone profile (Pampori

TABLE 1

Classification of sex-specific rat liver P450 genes and other genes

Genes are classified based on their response to hypophysectomy (Hx), pulsatile GH treatment of Hx rats, and continuous GH infusion in intact male rats. Male class II genes are expressed at high levels in both male and female Hx rats; they are subject to partial repression by male GH pulses (Wauthier and Waxman, 2008) and nearly complete repression by continuous GH treatment. Male class I genes, such as *CYP2C11*, are positively regulated by plasma GH pulses but are also repressed by continuous GH treatment, hence their increase in expression in Hx female liver to a level similar to that found in Hx male liver.

Gene Class	Prototypic Rat Genes	Loss of GH		GH Treatment	
		Male Hx	Female Hx	GH Pulses	GH Continuous
Male					
Class I	*CYP2C11*	↓	↑	↑	↓
Class II	*CYP2A2*, *CYP2C13*, *CYP3A2*	–	↑	↓	↓
Female					
Class I	*CYP2C12*, *HNF6*, *A1BG*	↓	↓	↓	↑
Class II	*ADH4*, *IGFBP1*	↑	↑	↓	↑

↑, increase in gene expression compared with untreated controls; ↓, decrease in gene expression compared with untreated controls; –, no major changes in expression.

and Shapiro, 1999). Male GH pulses also repress the expression of class II male P450 genes (Agrawal and Shapiro, 2000) in a process that is rapid (detected within 30 min), as revealed by analysis of the effects of a single short-term GH pulse on hepatic levels of the primary, unspliced transcripts (hnRNAs) of *CYP2A2* and *CYP2C13* in hypophysectomized male rat liver (Wauthier and Waxman, 2008). These latter findings suggest that the transcription of class II male-specific genes is restricted to the GH-free interpulse interval in adult male rat liver.

Female-specific genes can also be classified into sets of class I and II genes based on their response to hypophysectomy and GH replacement (Wauthier and Waxman, 2008). The steroid sulfate 15β-hydroxylase *CYP2C12* is a class I female-specific gene; it requires continuous exposure to GH to restore its expression in hypophysectomized rat liver (MacGeoch et al., 1985; Pampori and Shapiro, 1996). It can also be induced to near-female levels by continuous GH infusion in intact male rats (Fig. 1B and Table 1). *CYP2C12* is induced in intact female but not male rat liver at puberty, at the time when strong pituitary GH secretion commences, reflecting the requirement for continuous GH stimulation for high level expression (Fig. 1D). *ADH4* and *IGFBP1* are examples of class II female-specific genes: they are strongly derepressed (i.e., up-regulated) in both male and female liver after hypophysectomy, with a loss of sex specificity, and they are rapidly suppressed by physiological GH pulse replacement (Wauthier and Waxman, 2008). Continuous GH infusion in hypophysectomized female rats at doses that yield only ~3% of the mean female plasma GH concentration (~40 ng/ml) induces female P450 genes and suppresses both class I and II male P450 genes (Pampori and Shapiro, 1996). The effectiveness of such low GH concentrations is consistent with the low K_d for GH binding exhibited by GH receptor, ~2 ng/ml (Fuh et al., 1992), which corresponds to only ~1% of the peak plasma GH concentration in male rats (~ 200 ng/ml).

Sex differences in the intrinsic responsiveness of the liver to continuous GH are apparent, with the effects of continuous GH on liver P450 expression being less complete and/or requiring a higher GH dose in a male hypophysectomized rat model compared with a female hypophysectomized rat model (Pampori and Shapiro, 1999). These intrinsic sex differences in continuous GH responsiveness can be recapitulated in primary hepatocyte cultures (Thangavel et al., 2004). Likewise, male hepatocytes are more responsive than female hepatocytes to GH pulses applied in culture (Thangavel et al., 2006), which could reflect elevated levels of the JAK2-STAT5 signaling inhibitors CIS and SOCS2 (Ram and Waxman, 1999; Ram and Waxman, 2000) in the female-derived liver cells (Thangavel and Shapiro, 2007). Epigenetic mechanisms, such as DNA methylation and histone methylation, could underlie these sex differences, reflecting a memory of the sexual differentiation of intact liver in vivo. Alternatively, these sex differences could be genetically determined [e.g., by Y-chromosome-encoded genes, several of which show strong expression in liver (Clodfelter et al., 2006)] and could potentially modulate responsiveness to GH stimulation.

Requirement of STAT5b for the Sex-Dependent Actions of GH

GH activates multiple intracellular signaling pathways (Herrington and Carter-Su, 2001), one of which involves STAT5b, a GH-responsive transcription factor that has been implicated in many of the sex-dependent actions of GH on the liver (Waxman and O'Connor, 2006). GH signaling is initiated at the cell surface, where GH binds to its receptor and induces a conformational change that activates Janus kinase 2 (JAK2), a GH receptor-associated tyrosine kinase. The activated JAK2 phosphorylates GH receptor on several intracellular tyrosine residues, thereby forming docking sites for STAT5b and other downstream signaling molecules that have SH2 (phosphotyrosine-binding) domains. JAK2 subsequently phosphorylates the GH receptor-bound STAT5b on tyrosine 699, which leads to STAT5b dimerization and translocation to the nucleus, where the active STAT5b dimer binds DNA response elements containing the consensus sequence TTC-NNN-GAA and activates gene transcription (Darnell, 1997; Herrington and Carter-Su, 2001). STAT5b is one of

TABLE 2

Regulation of sex-specific genes in mouse liver by GH, STAT5b, and HNF4α

Data are based on Sakuma et al. (2002) and Holloway et al. (2006, 2007, 2008). Sex-specific mouse liver genes are classified based on the response to hypophysectomy (Hx), as described for rat liver (Wauthier and Waxman, 2008), with the male class I genes further divided into subclasses A, B, and C based on the impact of Hx in female liver, as shown in this table. The responses to Hx in male liver reflect ablation of the stimulatory effects (male class IA, IB, and IC genes) as well as the inhibitory effects (female class II genes) of both STAT5b and male GH pulses, which activate STAT5b. In male mice, STAT5b deficiency and HNF4α deficiency have essentially the same impact on sex-specific gene expression, suggesting that these two factors coregulate gene expression, as discussed in the text. "*Mups*" refers to several closely related *Mup* family genes. The *Cyp3a* genes (last column) do not respond to GH pulse treatment or STAT5b deficiency; they also respond unusually slowly ($\geq$7 days) to continuous GH treatment in intact male mice, indicating a unique regulatory mechanism. The response of *Cyp2d9* to STAT5b deficiency in female mice is based on analysis of hepatocyte-specific *Stat5a*/*Stat5b*-deficient mice (Holloway et al., 2007). .

	Male			Female	
	Class IA	Class IB	Class IC	Class II	Other
Prototypic mouse genes	*Cyp2d9*	*Cyp7b1*, *Mups*	*Cyp4a12*, *Gstp*	*Cyp2a4*, *Cyp2b9*	*Cyp3a16*, *Cyp3a41*,*Cyp3a44*
GH deficiency					
Hx Male	↓	↓	↓	↑	–
Hx Female	–	↓	↑	–	↓
GH treatment					
Hx Male + GH pulses	↑	↑	↑	↓	–
Male + continuous GH	↓	↓	↓	↑	↑
STAT5b deficiency					
Male	↓	↓	↓	↑	–
Female	↑	↓	–	–	–
HNF4α deficiency					
Male	↓	↓	↓	↑	–
Female	↓	↓	–	–	↓

↑, increase in gene expression compared with untreated controls; ↓, decrease in gene expression compared with untreated controls; –, no major changes in expression.

seven mammalian STAT transcription factors that share a conserved SH2-domain and a carboxyl-terminal tyrosine phosphorylation site (Grimley et al., 1999). STAT5a is closely related to STAT5b, with >90% sequence similarity, but is apparently unable to compensate for the loss of STAT5b in a gene knockout mouse model (Udy et al., 1997) and in humans with deleterious mutations in the *STAT5b* gene (Rosenfeld et al., 2007). STAT5a is essential for normal mammary gland development (Liu et al., 1997), whereas STAT5b is required for GH-stimulated pubertal and adult growth and for the sex-dependent effects of GH on liver gene expression (Udy et al., 1997; Teglund et al., 1998), as discussed below.

STAT5b is rapidly activated by each incoming plasma GH pulse in adult male rat liver (Fig. 2), whereas in adult female rats, liver STAT5b activity is maintained at a low but persistent level by the more continuous plasma GH pattern (Waxman et al., 1995; Choi and Waxman, 1999; Choi and Waxman, 2000). Hepatic STAT5b activity seems to have a similar sex dependence in mouse liver (Sueyoshi et al., 1999), although differences can be expected based on the unique features of the sexual dimorphic plasma GH profiles reported for mice (MacLeod et al., 1991). The sexual dimorphism of hepatic nuclear STAT5b activity, and its direct dependence on male plasma GH pulse stimulation, suggested that STAT5b might mediate the sex-dependent effects of GH on male liver gene expression (Waxman et al., 1995). Indeed, mice with a global disruption of the *Stat5b* gene display a loss of male-specific liver gene expression and lose their male-characteristic body growth rate profile (Udy et al., 1997; Teglund et al., 1998). Of seven male-specific liver RNAs investigated (Holloway et al., 2006), all were down-regulated (class I genes; Table 2), whereas five of eight female-specific RNAs were up-regulated in male liver in the absence of STAT5b (class II genes; Table 2) (Holloway et al., 2006). These findings are supported by a genome-wide transcriptional profiling study, where 90% of 850 male-specific genes were down-regulated and 61% of 753 female-specific genes were up-regulated in male liver in the absence of STAT5b (Clodfelter et al., 2006). In contrast, 90% of the sex-dependent genes investigated were unaffected by the loss of STAT5b in female liver, where STAT5b activity is much lower than in male liver (Choi and Waxman, 1999), and where STAT5a is required for expression of a subset of female-specific genes (Clodfelter et al., 2007). Thus, in male liver, STAT5b maintains male liver gene expression through its stimulatory effects on male-specific genes and its inhibitory effects on female-specific genes. These effects of STAT5b are likely to involve a combination of direct and indirect regulatory mechanisms, as discussed below.

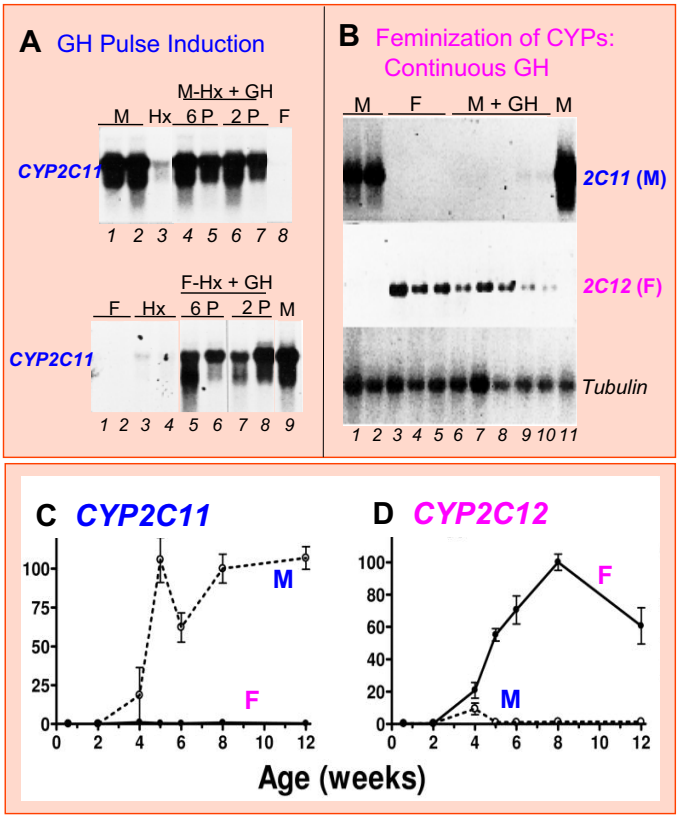

Fig. 1. Regulation of rat liver *CYP2C11* (male-specific) and *CYP2C12* (female-specific) by plasma GH profile (A and B) and through postnatal development (C and D). A and B, Northern blots probed for the indicated liver mRNAs isolated from intact male (M) and female (F) rats, or from hypophysectomized (Hx) rats given physiological GH pulses (P) by intravenous injection six or two times per day for 7 days (A; data from Waxman et al., 1991). GH pulse treatment induces *CYP2C11* in both male (top) and female (bottom) rats. Intact male rats were infused with GH continuously for 7 days using an osmotic mini-pump (B; data from Sundseth and Waxman, 1992), which suppresses *CYP2C11* and induces *CYP2C12*. Tubulin RNA serves as a loading control. Each lane represents an individual rat liver. C and D show rat hepatic mRNA levels for *CYP2C11* and *CYP2C12* at various postnatal ages, as determined by quantitative polymerase chain reaction (data from Laz et al., 2007).

Potential Limitations of the Global STAT5b-Deficient Mouse Model

The near-global effect of *Stat5b* gene disruption on sex-specific liver gene expression could be a direct result of impaired GH signaling due to the loss of STAT5b per se. However, indirect mechanisms involving the neuroendocrine control of pituitary GH secretion could also explain the observed demasculinization of male liver in the absence of STAT5b. GH secretion by the anterior pituitary is controlled by the action of two hypothalamic hormones: somatostatin, which inhibits GH release, and GH-releasing hormone, which stimulates GH secretion (Hartman et al., 1993; Fodor et al., 2006). Somatostatin mRNA is down-regulated in the hypothalamus of STAT5b-deficient mice, suggesting a role for hypothalamic STAT5b in the feedback regulation of somatostatin neurons by GH (Bennett et al., 2005). Consequently, the inhibition of pituitary GH release by somatostatin could be impaired in STAT5b knockout mice in a manner that feminizes circulating GH patterns and liver gene expression. A second possibility is that the loss of sex-specific liver gene expression in STAT5b-deficient male liver is a consequence of the down-regulation of *Igf1*, a STAT5b-dependent gene (Davey et al., 2001), the deletion of which in liver increases circulating GH levels as a result of the loss of a feedback inhibitory mechanism that regulates pituitary GH release (Sjögren et al., 1999; Wallenius et al., 2001). Indeed, circulating GH levels are elevated in global STAT5b knockout mice (Udy et al., 1997), and this could contribute to the feminization of male liver in the absence of STAT5b.

The impact of STAT5b deficiency on liver *Cyp* expression has been investigated in hypophysectomized mice given exogenous pulses of GH to help distinguish direct effects of STAT5b deficiency in the liver from indirect effects that the loss of STAT5b in the hypothalamus (or decreased *Igf1* ex-

pression in the liver) may have on liver gene expression as a result of changes in the hypothalamic regulation of pituitary GH secretion. Whereas GH pulse treatment of hypophysectomized mice restored male liver gene expression and male-characteristic body growth rates in wild-type mice, these responses were not seen in STAT5b knockout mice (Davey et al., 1999; Holloway et al., 2006). This indicates a requirement for STAT5b for GH pulses to stimulate sex-specific gene expression in male liver. In other studies, hepatocyte-specific deletion of *Stat5b* and the neighboring *Stat5a* gene using a *Cre* transgene under the control of the *albumin* promoter significantly decreased liver expression of male-specific P450 genes and other genes and concomitantly increased female-specific liver gene expression in male mouse liver (i.e., a pattern very similar to that of the global STAT5b knockout) (Holloway et al., 2007). Although plasma GH levels were elevated in individual hepatocyte STAT5a/STAT5b-deficient male mice, the maintenance of normal male body growth rates and the absence in these male mice of certain female-specific, continuous GH-inducible liver gene transcripts (e.g., *Cyp3a16*; Holloway et al., 2007) indicates that the loss of STAT5b, and not the increase in plasma GH levels, is responsible for the observed demasculinization of liver gene expression. Targeted disruption of the *Stat5a* gene alone has a greater impact on sex-specific gene expression in female liver than in male liver (Clodfelter et al., 2007; Holloway et al., 2007), supporting the conclusion that the loss of STAT5b per se is responsible for the feminization of male liver gene expression in these STAT5-deficient mouse models.

STAT5-Dependent Sex Differences in Postnatal Growth

In addition to the above-described affects of GH on liver gene expression, pituitary GH release imparts sex-differences to long bone growth and overall body growth rates. These sex differences become apparent at puberty, when the sex differences in plasma GH patterns first emerge (Pampori et al., 1991; Gabriel et al., 1992). The male-characteristic pubertal body growth spurt is greatly reduced in global *Stat5b*-disrupted mice, resulting in decreased growth (Udy et al., 1997; Teglund et al., 1998), similar to that seen in STAT5b-deficient humans (Hwa et al., 2005; Vidarsdottir et al., 2006) and certain models of liver STAT5 deficiency (Engblom et al., 2007). Other studies, however, suggest that normal postnatal growth requires STAT5b signaling in one or more extrahepatic tissues, such as bone and skeletal muscle (Klover and Hennighausen, 2007). Further insight into the role of STAT5 in postnatal growth is provided by mouse models in which the intracellular cytoplasmic tail of GH receptor (required for STAT5 signaling) has been deleted or the membrane-proximal "Box 1" sequence of GH receptor (which binds JAK2) has been mutated to abolish JAK2 binding and thereby eliminate GH signaling via STAT5 and other molecules (Rowland et al., 2005; Lichanska and Waters, 2008). The requirement of glucocorticoids for postnatal growth may also be explained by their role in coregulating a subset of STAT5b-dependent transcripts (including some sex-specific transcripts) via a glucocorticoid receptor-STAT5b transcriptional complex (Engblom et al., 2007).

Impact of Liver-Specific Hnf4α-Deletion on Sex-Specific Gene Expression

HNF4α (NR2A1) is a member of the nuclear receptor superfamily (Sladek, 1994; Gonzalez, 2008). It is essential for normal liver development (Lemaigre and Zaret, 2004) and regulates multiple liver functions, including lipid homeostasis, lipoprotein production, and bile acid biosynthesis (Hayhurst et al., 2001; Hanniman et al., 2006; Inoue et al., 2006; Miura et al., 2006). Many sex-specific hepatic genes require HNF4α for full expression, as evidenced by the functional binding sites for HNF4α found in the promoters of several sex-specific P450 genes, including mouse *Cyp2a4*, mouse *Cyp3a16*, and rat *CYP2C12* (Yokomori et al., 1997; Sasaki et al., 1999; Nakayama et al., 2001). In humans, a single nucleotide polymorphism at a putative HNF4α binding site in the *CYP2B6* gene has been linked to polymorphic gene expression (Lamba et al., 2003), and in primary human hepatocytes, HNF4α is required for the expression of *CYP3A4*, *CYP3A5*, and *CYP2A6* (Jover et al., 2001).

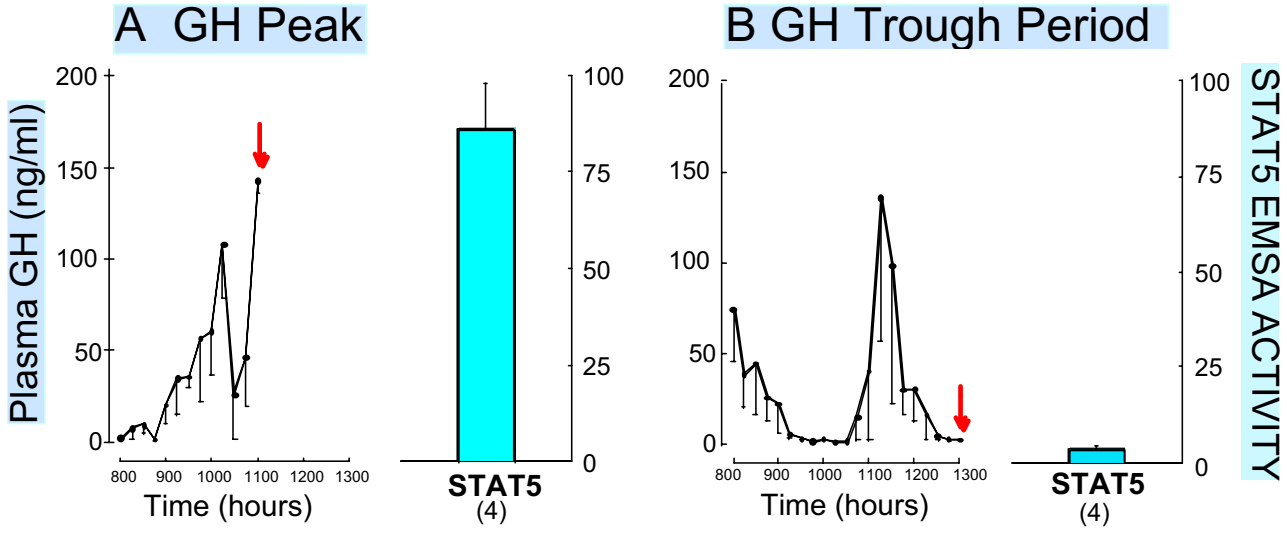

Fig. 2. Liver STAT5 is activated in direct response to each plasma GH pulse in adult male rats. Plasma samples obtained from intact rats every 15 min were assayed for GH. Samples were collected from 8 to 11 AM (A) or from 8 AM to 1 PM (B), with the collection end times of each group of n = 4 rats corresponding to a peak (A) and a trough in plasma GH levels (B), as indicated. The rats were then killed (red arrow) and the livers were assayed for STAT5 DNA-binding activity (blue bar on right). Data shown are from Tannenbaum et al. (2001).

Liver-specific deletion of the *Hnf4α* gene (Hayhurst et al., 2001) has a sex-dependent impact on gene expression in liver, with ~1000 fewer genes responding to the loss of HNF4α in female compared with male mice (Holloway et al., 2008). The male-predominant impact of liver HNF4α deficiency is even more striking when sex-specific genes are considered (372 sex-specific genes are specifically affected by the loss of HNF4α in male mouse liver, versus only 61 sex-specific genes are specifically affected in female mouse liver). The similarity between the response of sex-specific liver genes to the loss of HNF4α compared with the loss of STAT5b in male liver is striking, with parallel responses seen for 65% of the sex-specific genes common to the wild-type mouse strains used in both knockout studies (Table 2) (Clodfelter et al., 2006; Holloway et al., 2008). Thus, in male mouse liver, STAT5b and HNF4α positively regulate many male-specific *Cyp* genes and at the same time suppress a subset of female-specific genes. Moreover, the impact of STAT5b deletion and HNF4α deletion on sex-specific gene expression is substantially reduced in female compared with male liver. Thus, STAT5b and HNF4α may coregulate sex-specific liver genes by mechanisms that are primarily active in male liver. STAT5b and HNF4α exhibit mutual signaling cross-talk (Park et al., 2006); STAT5b is able to enhance HNF4α activation of select male-specific liver P450 genes, including *Cyp2d9* and *CYP8B1* (Wiwi and Waxman, 2005). STAT5b and HNF4α binding sites are found in the promoter regions of several sex-specific *Cyp* genes, including *Cyp2d9* and *Cyp2a4* (Yokomori et al., 1997; Wiwi et al., 2004). Moreover, *HNF6* (*ONECUT1*), a female-predominant transcription factor (Lahuna et al., 1997) that positively regulates the female-specific genes *CYP2C12* and *A1BG* (Delesque-Touchard et al., 2000; Gardmo and Mode, 2006), shows increased 5′-regulatory region binding of STAT5b and HNF4α in liver cells stimulated with GH (Lahuna et al., 2000).

A substantial fraction (31%) of the liver-expressed genes that contain functional HNF4α binding sites within 10 kilobases of the transcription start site, as determined by chromatin immunoprecipitation (Odom et al., 2007), require HNF4α for expression in one or both sexes (Holloway et al., 2008). The set of HNF4α-bound genes is 5-fold enriched for genes that are positively regulated by HNF4α (Holloway et al., 2008), supporting the conclusion that positive regulation by HNF4α proceeds by a direct DNA-binding mechanism. HNF4α protein and DNA-binding activity (but not RNA levels) are ~5-fold higher in male compared with female rat liver (C. A. Wiwi and D. J. Waxman, unpublished observations), which suggests a mechanism for the sex specificity that characterizes certain HNF4α-dependent genes.

Mechanisms Whereby STAT5b Regulates Sex-Specific Gene Expression

Given the stimulatory effects of liver STAT5b on male-specific gene expression established by the mouse knockout studies discussed above, STAT5b may directly *trans*-activate its male-specific gene targets (Fig. 3). This mechanism is likely to apply to those male-specific genes that are rapidly activated in liver in vivo in response to a single plasma GH pulse, such as the *Mup* genes (Wauthier and Waxman, 2008). Indeed, *Mup* genes bind STAT5 at multiple sites in male liver chromatin, as determined by chromatin immunoprecipitation, with no specific binding to these sites detectable in female liver (Laz et al., 2009). STAT5 binding to liver chromatin is dynamic; i.e., it is directly induced by GH and cycles on and off chromatin in response to each plasma GH pulse. Male-specific STAT5 binding in liver in vivo is observed at low-affinity STAT5 binding sites, including those associated with *Mup* genes (Laz et al., 2009). The persistent but low level of STAT5 typically found in female liver nuclei (Choi and Waxman, 1999) is too low for efficient binding to occur at these sites. In contrast, STAT5 binds to chromatin at similar levels in male and female liver at several high-affinity STAT5 sites implicated in the regulation of GH-responsive genes such as *IGF1* and *SOCS2* (Laz et al., 2009), which do not show marked sex specificity. Based on these findings, it can be predicted that male-specific genes that are directly regulated by STAT5b (e.g., *Mup* genes) will have low-affinity STAT5 binding sites. These may potentially include nonconsensus STAT5 sites (Soldaini et al., 2000), which could complicate efforts aimed at their discovery (Park and Waxman, 2001).

Two findings indicate that for a majority of liver male-specific genes, the requirement for STAT5b for full expression in male liver is likely to involve an indirect mechanism.

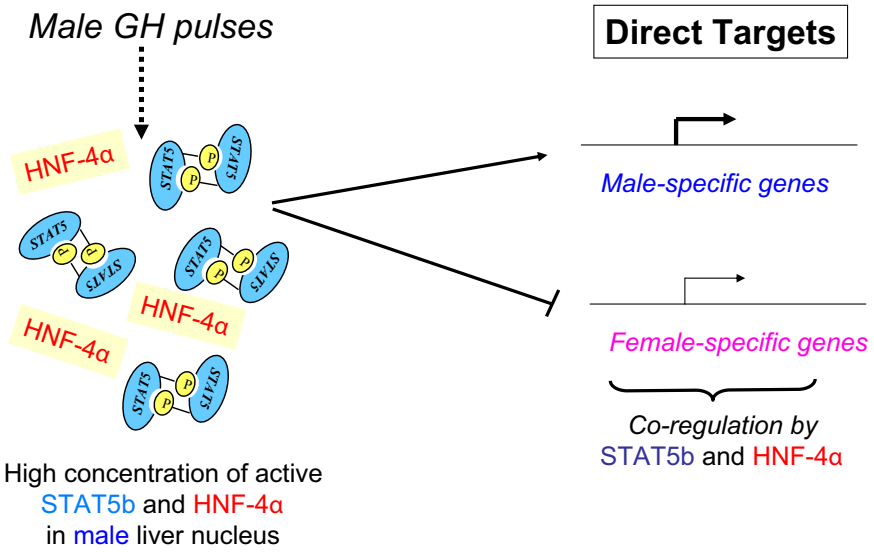

Fig. 3. Model for direct regulation of sex-specific genes by STAT5b and HNF4α in male liver. In the model shown, GH pulse-activated STAT5 binds directly to and *trans*-activates its direct male-specific genes targets, which may include the family of male-specific *Mup* genes (Laz et al., 2009). HNF4α may also contribute to the expression of these genes. STAT5 and HNF4α may also directly bind to and repress female-specific genes, as shown. Some of the genes that serve as primary targets of STAT5 and HNF4α may also mediate the effects of these transcription factors on their downstream (i.e., indirect) targets, as shown in Fig. 4.

First, most sex-specific genes are not activated when a single physiological plasma GH pulse is given to hypophysectomized animals (Wauthier and Waxman, 2008), even though liver STAT5 activation is rapid (within 5–10 min) under these conditions (Ram et al., 1996); second, continuous GH infusion in male mice feminizes liver gene expression slowly (over a period of days) (Holloway et al., 2006), whereas the down-regulation of STAT5 signaling occurs rapidly (within a few hours) (Fernández et al., 1998; Gebert et al., 1999). In one model of indirect regulation, a limited number of transcriptional regulators or other signaling molecules serve as the primary targets of GH pulse-activated STAT5b. These include transcriptional activators and transcriptional repressors, which regulate a larger set of secondary sex-specific target genes through a hierarchical network that includes both positive and negative regulatory mechanisms (Fig. 4). In an alternative formulation of this model, activation of some male-specific genes could require the coordinate binding of STAT5b and a STAT5b-dependent transcriptional activator. Several candidates for these primary transcriptional regulators have been identified based on their early response to a single plasma GH pulse given to hypophysectomized rats (Wauthier and Waxman, 2008). Liver expression of these primary response factors could be subject to postnatal developmental control that requires factors distinct from STAT5b. This, in turn, could help explain the finding that liver STAT5 alone is not sufficient to induce an adult male pattern of liver gene expression when it is activated precociously in the livers of prepubertal rats given exogenous GH pulses (Choi and Waxman, 2000). Indirect GH regulation of female-specific genes is also likely, as suggested by studies of the GH-regulated and female-predominant transcriptional activators HNF6 (Onecut1), HNF3α (Foxa1), and HNF3β (Foxa2), which can *trans*-activate female-specific genes, such as *CYP2C12* and *A1BG* (Delesque-Touchard et al., 2000; Gardmo and Mode, 2006) and *Cyp2b9* (Hashita et al., 2008). Finally, three nuclear factors are derepressed in STAT5b-deficient male liver and also HNF4α-deficient male liver; all three factors (Cux2/Cutl2, Tox, and Trim24) are female-specific transcriptional repressors that could contribute to the global down-regulation of male-specific genes seen in STAT5b-deficient male liver (Laz et al., 2007).

Although STAT5b is not generally thought of as a transcriptional repressor, widespread derepression of female-specific genes is observed in both hypophysectomized and STAT5b-deficient male mouse liver (class II female genes; Table 2) (Clodfelter et al., 2006), as noted above. STAT5b could mediate the inhibitory effects of plasma GH pulses on these female-specific genes, and on class II male-specific genes in male liver (Wauthier and Waxman, 2008), in an indirect manner, as exemplified by its inhibition of IGF binding protein-1 expression through effects on the transcription factor FoxO1 (Ono et al., 2007). Twice as many genes were repressed by STAT5b as were induced in studies where adenoviral vectors were used to deliver a constitutively active form of STAT5b to the liver, suggesting that STAT5b is a major mediator of the rapid suppressive effects of GH (Vidal et al., 2007). It has been suggested that even the rapid transcriptional effects of GH in the liver largely proceed via STAT5b-independent pathways, given the lack of phylogenetically conserved STAT5b binding sites immediately upstream or within hepatic genes that are rapidly induced by GH treatment (Vidal et al., 2007). However, the involvement of far upstream STAT5 sites, not examined in that study but seen in the case of *Igf1* (Wang and Jiang, 2005; Laz et al., 2009), cannot be excluded.

Hepatic STAT5a/STAT5b deficiency leads to aberrant activation of two other STAT family members, STAT1 and STAT3 (Cui et al., 2007), a response also seen in both male and female livers of global STAT5b knockout mice (Udy et al., 1997). Because each STAT protein regulates its own unique set of target genes, the activation of STAT1 and STAT3 in STAT5-deficient liver is likely to lead to changes in the expression of genes that are otherwise independent of STAT5b. Thus, some of the genes that are up-regulated in both male and female global STAT5b-deficient male mice (Clodfelter et al., 2006) may be induced due to the increased activity of STAT1 and STAT3, rather than the loss of inhibitory signaling by STAT5b per se (Hosui and Hennighausen, 2008).

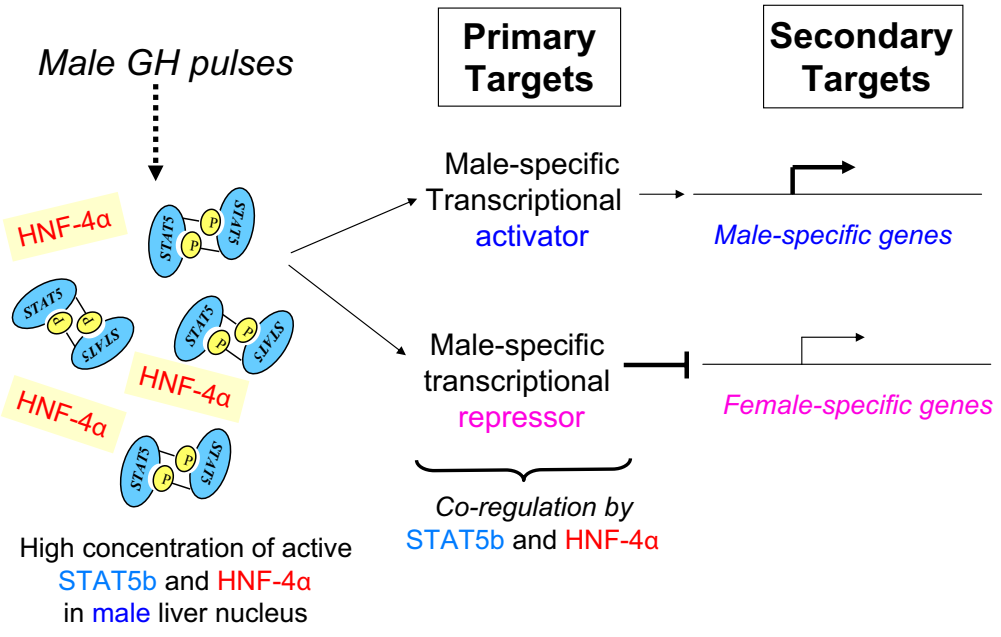

Fig. 4. Model for indirect regulation of sex-specific genes by STAT5b and HNF4α in male liver. In the model shown, the effects of STAT5b and HNF4α on sex-specific liver gene expression are mediated indirectly, by male-specific transcriptional activators and repressors, which are primary targets of these two transcription factors. In an alternative formulation of this model (not shown), *trans*-activation of a secondary male-specific target gene could involve direct binding of STAT5b *and* the primary male-specific transcriptional activator. Several candidate primary target transcriptional regulators have been identified (Wauthier and Waxman, 2008).

Epigenetic Regulation of GH-Responsive Sex-Specific Genes

For many genes, a change in chromatin accessibility is an important, underlying mechanism of transcriptional control. Long-term silencing of gene expression by the conversion of euchromatin to heterochromatin involves epigenetic events, such as DNA CpG methylation, binding of sequence-specific repressors linked to recruitment of heterochromatin protein 1 and interactions with noncoding RNAs (Kwon and Workman, 2008; Delcuve et al., 2009) and plays a major role in tissue-specific gene expression. Support for a role of epigenetic factors in the regulation of sex-specific liver gene expression comes from the discovery of sex- and GH-dependent DNase I hypersensitive sites in the promoter regions of *CYP2C11* and *Slp*, which correlate with the male-specific expression of these genes (Hemenway and Robins, 1987; Ström et al., 1994), and from studies of female-specific hypersensitivity sites in the case of *CYP2C12* (Endo et al., 2005). These GH- and sex-regulated changes in chromatin accessibility could be functionally linked to the sexual dimorphism of gene expression in the liver and may be a general feature of sex-specific genes.

The slow feminization of gene expression in male liver after continuous GH infusion (Holloway et al., 2006) may involve large-scale chromatin remodeling that is ultimately dependent on either STAT5b or HNF4α. The silencing of male-specific genes after continuous GH treatment may require the local conversion of euchromatin to heterochromatin, whereas the de-repression of many female-specific genes that is seen in male liver deficient in STAT5b or HNF4α, or after continuous GH infusion, may require the conversion of heterochromatin to euchromatin (Fig. 5). The latter process could be facilitated by HNF3β (FOXA2), which is more highly expressed in female than male liver (Wiwi et al., 2004), and whose related family member, HNF3α (FOXA1), serves as a "pioneer factor" that binds to nucleosomal DNA at distant enhancers and facilitates recruitment of other tissue-specific factors (Lupien et al., 2008).

An unusually long period of continuous GH treatment ($\geq$7 days) is required to induce hepatic expression of the female-specific genes *Cyp3a16*, *Cyp3a41*, and *Cyp3a44* in adult male mouse liver (Fig. 6) (Holloway et al., 2006, 2007). These three female-specific genes can be induced in male liver by continuous GH treatment, even in the absence of STAT5a and STAT5b (Table 2) (Holloway et al., 2006, 2007), indicating a unique regulatory mechanism. This could involve STAT5-independent signaling pathways induced by GH, for example extracellular signal-regulated kinase/mitogen-activated protein kinase signaling (Ceseña et al., 2007), which requires a Src-like tyrosine kinase, rather than JAK2 signaling (Rowlinson et al., 2008). All three *Cyp3a* genes are expressed in both male and female liver before puberty, after which they are selectively down-regulated in male liver (Sakuma et al., 2002; Cheung et al., 2006). The silencing of these genes in male mouse liver at puberty could be associated with formation of a "permanent" closed heterochromatin structure; accordingly, their delayed responses to a change in plasma GH status may reflect molecular events required to reverse long-term silencing via CpG demethylation, the reversal of heterochromatin protein 1 binding (Kwon and Workman, 2008; Delcuve et al., 2009), and/or nucleosome displacement, processes that may be dependent on hepatocyte division associated with continued body growth and an increase in liver size.

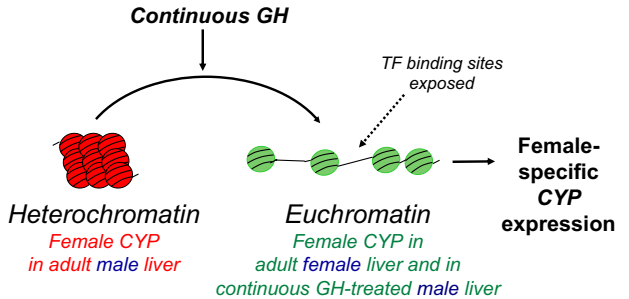

Fig. 5. Epigenetic regulation of sex-specific P450 genes. Female-specific *Cyp* genes are proposed to be repressed in male liver, and male-specific *Cyp* genes are proposed to be repressed in female liver by packaging in heterochromatin. Continuous GH is proposed to activate female-specific genes, such as *Cyp3a* genes, by a mechanism that involves the local conversion of heterochromatin to euchromatin, which enables the binding of transcription factors (TF) that activate P450 gene expression. This process could involve loss of DNA CpG methylation and/or loss of chromatin marks associated with repressed chromatin, such as histone H3 lysine 27 trimethylation, which is typically found in genes in a compact chromatin structure and is associated with a stable, inactive heterochromatic state (Cheung and Lau, 2005).

Conclusion

Studies carried out in rat and mouse models have established that GH is the key hormonal factor that dictates sex differences in the expression of a large number of liver gene products, including many P450s and other DMEs. A similar mode of regulation may characterize CYP3A4 and other DMEs in humans, where clinically significant sex differences in metabolism are increasingly being recognized. GH is proposed to activate a complex hierarchical regulatory network of transcription factors that exert both stimulatory and inhibitory effects on sex-specific DMEs and other liver-expressed genes. The transcription factors STAT5b and HNF4α

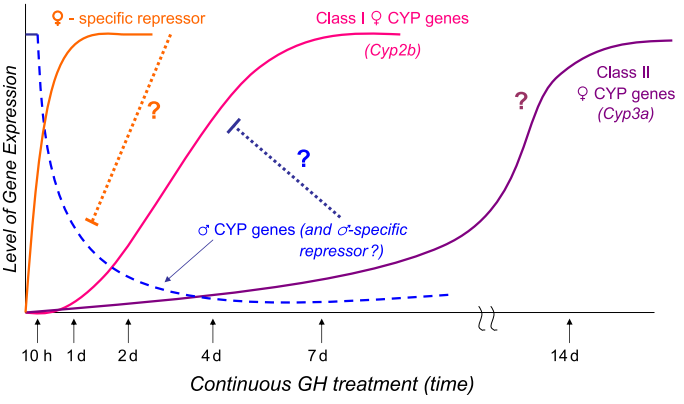

Fig. 6. Time course for continuous GH feminization of P450 gene expression in adult male liver. Model shown is based on data presented in Holloway et al. (2006); hypothetical regulatory interactions are indicated by question marks. Female-specific repressors (Laz et al., 2007), are rapidly induced in male liver by continuous GH treatment and are proposed to down-regulate male-specific genes. The latter genes are proposed to include one or more male-specific repressors, whose down-regulation leads to de-repression of female-specific genes, such as *Cyp2b9* and *Cyp2b10*; these *Cyp2b* genes require several days of continuous GH treatment for induction (Holloway et al., 2006). A distinctly longer time frame ($\geq$7 days) is required for derepression leading to induction of the female-specific *Cyp3a* genes, which may involve epigenetic mechanisms, as discussed in the text and diagrammed in Fig. 5.

are both essential for the sexual dimorphism of an unexpectedly large number of liver-expressed genes. The actions of these factors are likely to be mediated through the actions of secondary target genes, including other transcription factors and downstream signaling molecules. Further studies are needed to identify and characterize these secondary regulators, the mechanisms by which they are regulated by GH and its sex-dependent plasma profiles, and their potential to contribute to sex-dependent chromatin remodeling and epigenetic events likely to be important in enforcing liver sex-specificity.

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