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Distinctive Roles of STAT5a and STAT5b in Sexual Dimorphism of Hepatic P450 Gene Expression

IMPACT OF *Stat5a* GENE DISRUPTION*

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***Stat5b* gene disruption leads to an apparent growth hormone (GH) pulse insensitivity associated with loss of male-characteristic body growth rates and male-specific liver gene expression (Udy, G. B., Towers, R. P., Snell, R. G., Wilkins, R. J., Park, S. H., Ram, P. A., Waxman, D. J., and Davey, H. W. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 7239–7244). In the present study, disruption of the mouse *Stat5a* gene, whose coding sequence is ~90% identical to the *Stat5b* gene, resulted in no loss of expression in male mice of several sex-dependent, GH-regulated liver cytochrome P450 (CYP) enzymes. By contrast, the loss of STAT5b feminized the livers of males by decreasing expression of male-specific CYPs (CYP2D9 and testosterone 16α-hydroxylase) while increasing to female levels several female-predominant liver CYPs (CYP3A, CYP2B, and testosterone 6β-hydroxylase). Since STAT5a is thus nonessential for these male GH responses, STAT5b homodimers, but not STAT5a-STAT5b heterodimers, probably mediate the sexually dimorphic effects of male GH pulses on liver CYP expression. In female mice, however, disruption of either *Stat5a* or *Stat5b* led to striking decreases in several liver CYP-catalyzed testosterone hydroxylase activities. *Stat5a* or *Stat5b* gene disruption also led to the loss of a female-specific, GH-regulated hepatic CYP2B enzyme. STAT5a, which is much less abundant in liver than STAT5b, and STAT5b are therefore both required for constitutive expression in female but not male mouse liver of certain GH-regulated CYP steroid hydroxylases, suggesting that STAT5 protein heterodimerization is an important determinant of the sex-dependent and gene-specific effects that GH has on the liver.**

The cytochrome P450s (CYPs)[1] are a superfamily of heme proteins that hydroxylate steroid hormones and other endogenous chemicals as well as numerous drugs and environmental carcinogens. CYPs are highly expressed in liver, where they are subject to complex hormonal regulation and sex-dependent expression. Prototypic examples of sex-specific liver CYPs in the rat model are the male-specific CYP2C11 (testosterone 16α- and 2α-hydroxylase) and the female-specific CYP2C12 (steroid sulfate 15β-hydroxylase) (1). Marked sex-dependent differences in hepatic CYP profiles are also seen in the mouse, where CYP2D9 (a testosterone 16α-hydroxylase) and CYP2A4 (a testosterone 15α-hydroxylase) are expressed in males and females, respectively, in certain strains (2, 3). Sexually dimorphic expression of a mouse CYP2B testosterone 16α-hydroxylase has also been described (4–6). The expression of these sexually dimorphic liver steroid hydroxylase CYPs is primarily determined by the sexual dimorphism of plasma growth hormone (GH) profiles (2, 7–9). Intermittent plasma GH pulses, a characteristic of adult male rats, induce expression of male-specific CYP proteins and their associated steroid hydroxylase activities, while the near continuous presence of GH in the plasma of adult female rats induces expression of female-specific and female-dominant liver CYP proteins (1, 10). The plasma GH pattern in mice is pulsatile in both sexes; however, sex-specific responses of liver CYPs to plasma GH profiles can be discerned by the distinct frequency of pulsation in males (interpulse interval of ~2.5 h) and females (interpulse interval <1 h) (11).

Pulsatile GH, but not continuous GH, strongly activates the signal transducer and transcriptional activator STAT5 in rat liver (12). STAT5 was originally identified in lactating mammary gland as a prolactin-inducible transcription factor (13). Subsequently, two highly conserved (~90% identical in coding sequence) STAT5 genes, *Stat5a* and *Stat5b*, were identified and found to be expressed in many tissues (14–17). Both STAT5 forms can be activated in tissue culture by multiple cytokines and growth factors, including GH, erythropoietin, epidermal growth factor, and various interleukins (14, 18–21). STAT5 proteins thus have the potential to contribute to multiple signaling pathways associated with cell growth and differentiation. The proposed mediation by STAT5 of GH pulse-regulated, sexually dimorphic liver gene expression (12) is supported by the finding of a functional STAT5 response element in the promoter region of several male-specific, GH pulse-regulated genes (22).[2] In addition, targeted disruption of *Stat5b* leads to a major loss of multiple, sexually differentiated responses associated with pulsatile GH secretion (23), demonstrating that this GH pulse-activatable transcription factor (12) is essential for maintaining sexually dimorphic body growth rates and liver gene expression.

During mammary gland differentiation, STAT5a and STAT5b both undergo prolactin-inducible tyrosine phosphorylation and bind as a heterodimeric STAT5a-STAT5b complex at γ-interferon activation-like regulatory sites (TTCNNNGAA), such as that found in the mouse β-casein promoter (13, 24).

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[1] The abbreviations used are: CYP, cytochrome P450; STAT, signal transducer and activator of transcription; EMSA, electrophoretic mobility shift assay; PCR, polymerase chain reaction; GH, growth hormone; bp, base pair(s).

[2] S. H. Park and D. J. Waxman, unpublished observations.

Stat5a gene disruption has demonstrated that STAT5a is critically required to activate and/or repress yet unknown target genes that promote mammary gland differentiation and lactogenesis (25). Mammary gland STAT5b can only partially fill this function and then again only after repeated hormonal stimulation through multiple pregnancies and episodes of suckling (26). Although targeted disruption of *Stat5b* leads to the loss of multiple sexually differentiated responses governed by a pulsatile plasma GH profile (23), it is uncertain whether this loss reflects a requirement for heterodimeric STAT5a-STAT5b complexes or perhaps an absolute requirement for homodimeric STAT5b-STAT5b complexes for GH pulse-regulated liver gene expression. STAT5a and STAT5b exhibit differences with respect to their tissue distribution (14, 15) and DNA binding specificities (27) and in the sequences of their COOH-terminal transcription activation domain (28). These two STATs could thus have distinct functions with respect to their role in GH signaling and its impact on the sexual dimorphism of liver gene expression.

To address the role of STAT5a in the GH-regulated sexual dimorphism of liver gene expression, we have examined the effects of *Stat5a* gene disruption on hepatic CYP enzyme activities and protein expression. 129J congenic *Stat5b*$^{-/-}$ mice were also examined to verify and extend using an inbred mouse strain our earlier findings (23) on the effects of *Stat5b* gene disruption in 129J × BALB/c outbred mice. Evidence is presented in support of the hypothesis that heterodimerized (STAT5a-STAT5b) and homodimerized (STAT5b-STAT5b) STAT5 complexes play distinct roles in the sexually differentiated responses of the liver to GH.

MATERIALS AND METHODS

Animals—Generation of *Stat5a* and *Stat5b* gene-disrupted mice has been described (23, 25). In order to evaluate the effects of STAT5b deficiency in a congenic 129J background, chimeric male mice (derived from 129J ES cells) were mated with 129J females. The *Stat5b*$^{+/-}$ progeny were then bred to obtain the 129J congenic *Stat5b*$^{-/-}$ and *Stat5b*$^{+/+}$ mice used for these experiments. Experiments using *Stat5a*$^{-/-}$ mice were carried out using littermates obtained from 129J × Black Swiss outbred mice. In all cases, comparisons of wild-type and STAT5-deficient mice were made between littermates obtained from the crossing of heterozygote siblings or of heterozygote females with null male siblings, all containing the same 129J outcrossed background. Accordingly, strain-specific P450 allelic determinants are expected to segregate randomly in both wild-type and *Stat5* gene-disrupted mice. Efforts to obtain liver tissue from 129J congenic *Stat5a*$^{-/-}$ mice for direct comparison to the 129J congenic *Stat5b*$^{-/-}$ mice were unsuccessful, since these animals proved very difficult to breed. Although the possibility cannot formally be excluded that a 129J-derived regulatory determinant of liver CYP gene expression is tightly linked to the *Stat5* locus and contributes to some of the liver CYP profile differences between wild-type and *Stat5a*$^{-/-}$ 129J × Black Swiss mice, this is considered unlikely.

Preparation of Mouse Liver Homogenates, Cytosol, and Microsomes—Mouse liver tissues were snap-frozen in liquid nitrogen and stored at −80 °C until use. Liver (about 1 g) was homogenized in 10 ml of homogenizing buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, and 250 mM sucrose) with the addition of phosphatase inhibitors (1 mM sodium orthovanadate and 10 mM sodium fluoride) and a protease inhibitor (100 μM phenylmethanesulfonyl fluoride) and centrifuged at 9000 rpm for 15 min to obtain a total tissue homogenate. Ultracentrifugation at 100,000 × *g* for 1 h was carried out to separate microsomal pellets and the cytosolic supernatant. Microsomal pellets were suspended in KPi buffer, pH 7.4, containing 0.1 mM EDTA and 20% glycerol, and stored at −80 °C until use. Microsomal protein concentrations were determined using the Bradford assay kit (Sigma). Cytosolic and total tissue homogenate protein concentrations were determined using the Bio-Rad Dc detergent protein assay kit using bovine serum albumin as a standard.

Antibodies—Rabbit polyclonal antibodies raised against mouse STAT5a amino acids 774–793 and mouse STAT5b amino acids 776–786 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) (sc-1081 and sc-835, respectively). These STAT5 antibodies were shown to be specific for STAT5a or STAT5b, respectively, under our Western blotting conditions. Some cross-reactivity was apparent in electrophoretic mobility shift assay (EMSA) supershift analysis (see Fig. 2*B*). Mouse monoclonal anti-STAT1 and anti-STAT3 antibodies purchased from Transduction Laboratories were raised against STAT1 amino acids 591–731 (S21120) and STAT3 amino acids 1–178 (S21320), respectively. Anti-rat CYP3A (mouse monoclonal antibody 2-3-2) (29), rabbit polyclonal anti-rat CYP2B1 (30), and rabbit polyclonal anti-mouse CYP2D9 antibodies (31) were used for Western blot analysis of microsomal CYP proteins. Anti-mouse CYP2D9 (anti-C-P450$_{16\alpha}$) was generously provided by Dr. M. Negishi (NIEHS, National Institutes of Health, Research Triangle Park, NC).

Western Blotting—Liver cytosolic protein (40 μg) and microsomal protein (20 μg) prepared from 8–9-week-old wild-type *Stat5a*$^{-/-}$ or *Stat5b*$^{-/-}$ mice were electrophoresed through standard Laemmli SDS-polyacrylamide gels (10% gels for STAT protein separation and 8% gels for mouse microsomal CYP protein separation), transferred to nitrocellulose membranes, and then probed with anti-STAT or anti-CYP antibodies. Membranes were blocked for 1 h at 37 °C with 3% nonfat dry milk (Blotto) and 1% bovine serum albumin in a high Tween buffer (0.3% Tween 20 in phosphate-buffered saline) for anti-STAT1 and anti-STAT3 or with 2% Blotto and 2% bovine serum albumin in TST buffer (10 mM Tris-HCl, pH 7.5, 0.1% Tween 20, 100 mM NaCl) for probing with anti-STAT5a and anti-STAT5b. For microsomal CYP Western blotting, membranes were blocked with 3% Blotto and 1% bovine serum albumin in TST buffer. Incubations with primary antibody were carried out for 1 h at 37 °C at dilutions of 1:3000. Antibody binding was visualized on x-ray film by enhanced chemiluminescence using the ECL kit from Amersham Pharmacia Biotech. Nitrocellulose membranes were reprobed after stripping in 62.5 mM Tris-HCl, pH 7.6, 2% SDS, 50 mM 2-mercaptoethanol for 20 min at 50 °C. Results are presented in figures prepared from grayscale scans of portions of the x-ray films of each blot. Scans were obtained using a Cannon IX-4015 scanner outfitted with Ofoto scanning software.

Microsomal Testosterone Hydroxylation Assay—Microsomal metabolism of testosterone was assayed as described previously (32) using 25 μg of mouse liver microsomal protein incubated in 0.2 ml containing 50 mM Tris buffer, pH 7.6, 3 mM $MgCl_2$, and ^{14}C-labeled testosterone (10 nmol, ~100,000 cpm). Reactions were initiated by the addition of 0.36 mM NADPH and terminated 20 min later by the addition of 1 ml of ethyl acetate. Testosterone and hydroxytestosterone metabolites were extracted with ethyl acetate and then chromatographed on silica gel TLC plates developed in solvent A (methylene chloride/acetone (80:20, v/v)) and then solvent B (chloroform/ethyl acetate/ethyl alcohol (70:17.5:12.5, v/v/v)). TLC plates were exposed overnight to PhosphorImager plates followed by quantitation using a Molecular Dynamics PhosphorImager instrument and ImageQuant software (Sunnyvale, CA). In order to assess the homogeneity and identity of individual radiolabeled testosterone metabolites, radioactive spots of interest were cut from the aluminum-backed TLC plates, and the ^{14}C-labeled monohydroxytestosterone metabolites were then eluted with ethyl acetate. Unlabeled authentic hydroxytestosterone metabolites were then individually co-spotted with each of the unknown ^{14}C-metabolites to verify co-migration in two independent TLC solvent systems (32).

EMSA—Total liver homogenate protein (15 μg) was preincubated for 10 min at room temperature with 9 μl of gel mobility shift buffer (12.5 mM Tris-HCl, pH 7.5 containing 10 fmol of DNA probe, 2 μg of poly(dI-dC) (Boehringer Manheim), 5% glycerol, 1.25 mM $MgCl_2$, 625 μM EDTA, and 625 μM dithiothreitol). Double-stranded oligonucleotide probe containing the STAT5 response element of the rat β-casein promoter (nucleotides −101 to −80; 5′-GGA-CTT-CTT-GGA-ATT-AAG-GGA-3′) was ^{32}P-end-labeled on one strand using T4 kinase and then incubated with the protein sample for 20 min at room temperature and then 10 min on ice to stabilize the STAT5-DNA gel shift complex (33). For supershift analysis, an additional 10-min incubation in the presence of STAT antibodies was carried out after the addition of the labeled DNA probe. Samples were electrophoresed in a cold room through a nondenaturing polyacrylamide gel (5.5% acrylamide, 0.07% bisacrylamide) (National Diagnostics, Atlanta, GA) in 0.5 × TBE buffer (44.5 mM Trizma-base, 44.5 mM boric acid, 5 mM EDTA) following a 30-min preelectrophoresis step. After electrophoresis of the samples into the gel for 20 min at 120 V, the gel apparatus was moved to room temperature to increase the speed of protein migration. In some cases, the electrophoresis time was increased and/or the percentages of acrylamide and bisacrylamide gel were increased to 6.5% and 0.08%, respectively, to increase the resolution of the STAT5-containing EMSA complexes.

RNA Isolation and Reverse Transcription Polymerase Chain Reaction (PCR)—Total RNA was isolated from ~100 mg of mouse liver using TRIZOL Reagent (Life Technologies, Inc.). First strand cDNA was syn-

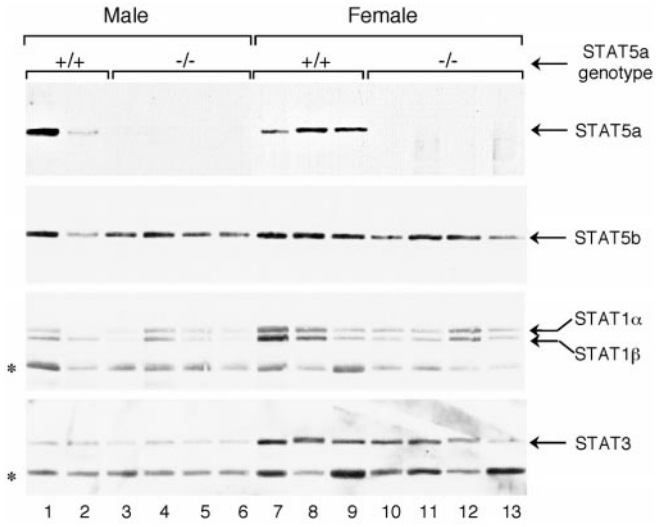

FIG. 1. **STAT proteins expressed in livers of *Stat5a*$^{-/-}$ mice.** Liver cytosols (40 μg) were prepared from individual male (*lanes 1–6*) and female (*lanes 7–13*) wild-type (*lanes 1*, *2*, and *7–9*) and *Stat5a* knock-out mice (*lanes 3–6* and *10–13*). Western blot analyses were carried out by sequentially probing with each of the indicated anti-STAT antibodies. Unidentified protein(s) cross-reactive with anti-STAT1 and STAT3 antibodies are marked by an *asterisk* at the *left*.

thesized using random hexamer by adding 7 μl of a master mix reaction buffer (1.5 mM $MgCl_2$, 15 mM KCl, 10 mM Tris-HCl, pH 8.3, 10 mM dNTPs, 0.1 M dithiothreitol) to 2 μg of RNA and 100 ng of random hexamer primer. Samples were incubated, first for 50 min at 42 °C and then for 15 min at 70 °C. RNase H (1 μl, 2 units) was then added, followed by a 30-min incubation at 37 °C. 2 μl of each cDNA sample was amplified in 50 μl of PCR reaction mixture (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 2 mM dNTPs, and 100 ng of each STAT5 PCR primer (see below)). Amplification was conducted in a Stratagene thermal cycler at 94 °C for 1 min, 55 °C for 1 min 10 s, and 72 °C for 1 min 30 s for 30 cycles. RNA that was not reverse transcribed was included as a negative control to verify the absence of genomic DNA or PCR product contamination. The efficiency of RNA extraction and of reverse transcription was determined by including β-actin-specific primers as an internal control in each PCR reaction. The STAT5 primers used in the experiment (forward primer, 5′-CAG GTG AAG GCG ACC ATC AT-3′; reverse primer, 5′-TG CTG TTG TAG TCC TCG AGG-3′) amplify both STAT5a and STAT5b cDNA to produce a 550-base pair PCR product using a protocol kindly provided by Dr. M. Negishi. To determine the relative STAT5a and STAT5b mRNA levels in mouse liver, the STAT5 PCR products were purified using a QIAquick PCR purification kit (QIAGEN, Valencia, CA) and then digested with *Nco*I restriction endonuclease (New England BioLabs, Beverly, MA). PCR-amplified STAT5a cDNA obtained using the above primers contains a single *Nco*I restriction site, which when cut yields fragments 240 and 310 bp in length, while PCR-amplified STAT5b cDNA does not contain the *Nco*I cutting site. Purified PCR products were digested with *Nco*I (2 units) in New England BioLabs buffer 4 for 2 h at 37 °C. PCR products and 1-kilobase pair molecular marker were electrophoresed on a 1.5% agarose gel and visualized by ethidium bromide staining, and their relative levels were quantitated using ImageQuant software.

Statistical Analysis—Individual group comparisons were performed using the two-tailed Student's *t* test ($p \leq 0.05$).

RESULTS

Stat5a Gene Disruption—Liver cytosols prepared from *Stat5a*$^{-/-}$ mice were assayed for the expression of individual STAT proteins by Western blotting. Wild-type mice showed similar levels of liver cytosolic STAT5a protein in both males and females, with some differences between individual animals apparent (Fig. 1, *lanes 1*, *2*, and *7–9*). No STAT5a protein was detected in the *Stat5a*$^{-/-}$ mice of either sex. Reprobing with antibodies to other STAT proteins revealed that STAT5b, STAT1, and STAT3 protein levels were not significantly changed by the *Stat5a* gene disruption. This contrasts to the increase in liver STAT1 in *Stat5b*$^{-/-}$ mice (23). STAT3 was present in liver at a significantly higher level in females than in males in the 129J × Black Swiss mice used in the present study (Fig. 1, *bottom panel*, *lanes 7–13 versus lanes 1–6*). No sex difference in hepatic STAT3 levels was seen, however, in 129J or in 129J × BALB/c mice (data not shown).

Our previous study of *Stat5b*$^{-/-}$ mice (23) was carried out using 129J × BALB/c outbred mice. Since there are significant strain differences in the patterns of CYP enzyme expression in mouse liver (4, 34, 35), we generated *Stat5b*$^{-/-}$ mice in the 129J inbred strain in order to eliminate random genetic contributions from the 129J × BALB/c outcrossed background to individual animal variation. Examination of the *Stat5b*$^{-/-}$ 129J congenic mice revealed effects of STAT5b disruption on liver STAT protein expression similar to those seen earlier in outbred mice; STAT1 levels were increased, while the expression of STAT3 and STAT5a was unchanged (data not shown).

Characterization of STAT5a and STAT5b DNA Binding Activity in Stat5 Gene-disrupted Mouse Liver—We next used a STAT5-binding DNA probe from the rat β-casein gene in an EMSA of total liver homogenates to examine the functional (DNA binding) activity of STAT5b protein in *Stat5a*$^{-/-}$ mice. STAT5 protein present in *Stat5a*$^{-/-}$ mouse liver was active in this DNA binding assay (Fig. 2*A*, *lane 1*). Given the absence of STAT5a protein in these livers, we conclude that the DNA complex detected is composed of STAT5b-STAT5b homodimers. These homodimers migrated distinctly faster than STAT5a homodimers, which are present in liver homogenates prepared from *Stat5b*$^{-/-}$ mice (*lanes 4* and *5*). A corresponding fast mobility complex was formed by a GH pulse-activated rat liver homogenate (*lane 8*), in agreement with earlier studies indicating that STAT5b is the major STAT5 protein in this tissue (36, 37). The major complex observed in wild-type mice migrated at an intermediate mobility compared with that present in *Stat5a*$^{-/-}$ and *Stat5b*$^{-/-}$ mice (*lanes 2*, *3*, *6*, and *7*), indicating the presence of STAT5a-STAT5b heterodimers.

The presence of STAT5 homo- or heterodimers in these liver samples was further investigated by supershift analysis using anti-STAT5a and anti-STAT5b antibodies (Fig. 2*B*). Anti-STAT5b antibody fully supershifted the STAT5b-containing DNA complex present in *Stat5a*$^{-/-}$ mice (Fig. 2*B*, *lane 9*). A supershift complex of similar mobility was obtained in wild-type mouse liver (*lanes 6* and *12*) and in male rat liver (*lane 3*), suggesting that these tissues contain homodimeric STAT5b-STAT5b complexes in addition to the intermediate mobility STAT5a-STAT5b heterodimeric complexes evident from Fig. 2*A*. Some cross-reactivity of this anti-STAT5b antibody with STAT5a-STAT5a homodimers was apparent, however, as revealed by the more rapidly migrating supershift complex obtained with *Stat5b*$^{-/-}$ mouse liver samples (*lane 15*). This latter complex was not present in significant amounts in wild-type mouse liver (*lanes 6* and *12*) or male rat liver (*lane 3*), indicating that the majority of activated STAT5a is complexed as a heterodimer with STAT5b in wild-type liver. Anti-STAT5a antibody yielded a nearly complete supershift of the STAT5a-STAT5a complexes present in *Stat5b*$^{-/-}$ mouse liver (*lane 14*) but only a partial supershift of the STAT5-containing complexes present in wild-type mice (*lanes 5* and *11*) or in male rat liver (*lane 2*). The supershift pattern obtained with anti-STAT5a antibody is consistent with the presence of both STAT5b-STAT5b homodimer and STAT5a-STAT5b heterodimer, as demonstrated for GH-activated STAT5a and STAT5b standards expressed in extracts of transfected COS-1 cells; STAT5a antibody completely supershifted STAT5a-STAT5a EMSA complexes, while it partially shifted STAT5a-STAT5b and STAT5b-STAT5b complexes formed by the transfected COS-1 cell extracts (data not shown). This cross-

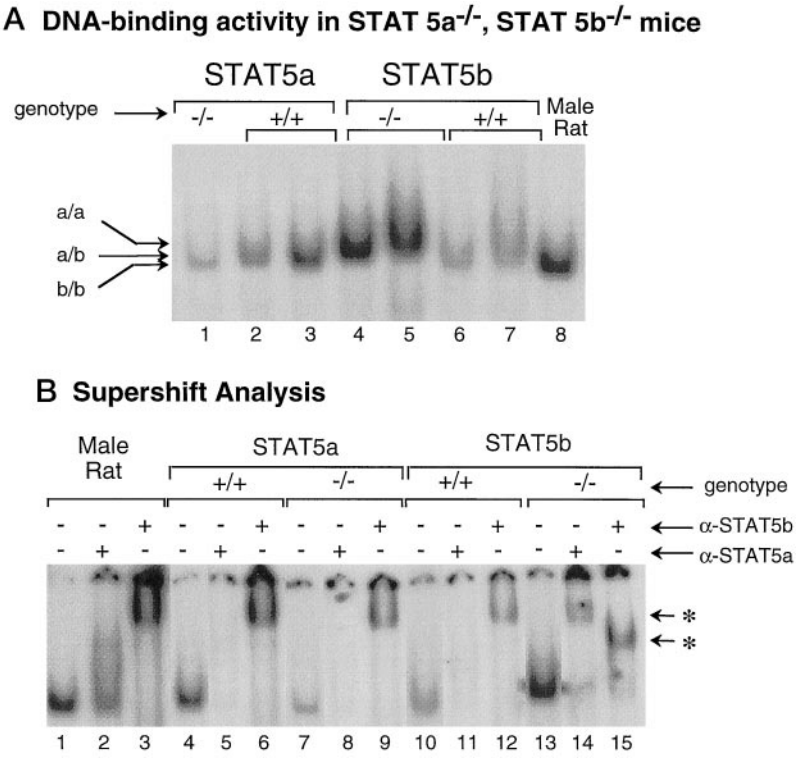

FIG. 2. **Gel shift analysis of liver STAT5 DNA binding activity from wild-type, *Stat5b*$^{-/-}$ and *Stat5a*$^{-/-}$ mice (*A*) in the absence or presence of supershifting anti-STAT5a and anti-STAT5b antibodies (*B*).** Liver extracts were prepared from individual mice and then analyzed by EMSA for STAT5 DNA binding activity as described under "Materials and Methods." *A*, a distinct mobility difference between STAT5a-STAT5a homodimers and STAT5a-STAT5b heterodimers is apparent from a comparison of *lanes 4* and *5 versus lanes 6* and *7*. A GH pulse-activated male rat liver extract is shown in *lane 8* for comparison. *B*, supershift analysis using anti-STAT5a and anti-STAT5b antibodies revealed some immune cross-reactivity between the STAT5a and STAT5b antibodies (see text). Male rat total liver homogenates were used as a positive control (*lanes 1–3*). *Lane 14* shows that the anti-STAT5a antibody can completely supershift the STAT5a-STAT5a homodimer that is present in the *Stat5b*$^{-/-}$ male mouse liver. *Arrows* with *asterisks* at the *right* mark supershifted protein-DNA complexes. Note distinct mobilities of each of the nonsupershifted complexes shown in *lanes 4*, *7*, *10*, and *13* as a function of the presence or absence of STAT5a or STAT5b (*cf. panel A*).

reactivity of anti-STAT5a with STAT5b was also apparent from the disruption of the STAT5b-STAT5b complex present in liver extracts from *Stat5a*$^{-/-}$ mice (*lane 8 versus lane 7*). Since STAT5 DNA binding activity is obligatorily dependent on tyrosine phosphorylation of the STAT protein (38), we conclude based on these data that STAT5a and STAT5b are both present in mouse liver in the activated form.

Expression of Sex-dependent CYPs in Stat5a$^{-/-}$ *Male Mice*—Experiments were carried out to ascertain whether STAT5a is required for expression of sex-dependent liver CYP enzymes. To achieve this objective, we first examined the patterns of CYP enzyme expression in male and female *Stat5a*$^{-/-}$ and *Stat5b*$^{-/-}$ mice, using the diagnostic CYP substrate testosterone (32). Liver CYP enzyme patterns are known to differ significantly between individual strains of mice (2, 34, 35). We observed several differences in the sex-dependence of liver microsomal testosterone hydroxylation in the two mouse strains used in this study, 129J × Black Swiss for *Stat5a*$^{-/-}$ and 129J inbred for *Stat5b*$^{-/-}$. Three female-dominant hydroxytestosterone metabolites were formed in wild-type 129J × Black Swiss liver microsomal incubations (2α-OH, 6α-OH (Fig. 3*A*) and 7α-OH (Table I)), while only a single female-dominant hydroxytestosterone metabolite (6β-OH; Fig. 3*B*) was formed by wild-type 129J mouse liver microsomes (Table I). In the case of 129J × BALB/c outbred mice, two female-dominant hydroxytestosterone metabolites (6α-OH and 6β-OH) were formed (Table I). In addition, 129J inbred mouse liver microsomes formed a male-specific 16α-OH-testosterone metabolite that was not observed in the 129J × BALB/c outbred mice used in our earlier *Stat5b*$^{-/-}$ studies (Fig. 3*B*; Table I). This latter strain difference is the result of a repression of a female-specific, CYP2B-dependent testosterone 16α-hydroxylase in 129J females (4).

In male 129J mice, the loss of STAT5b increased the female-dominant testosterone 6β-hydroxylase enzyme activity up to the level of wild-type females (Fig. 3*B*). The same response to the loss of STAT5b was seen for two CYP activities that have a female-dominant expression profile in 129J × BALB/c mice, testosterone 6α-hydroxylase and testosterone 6β-hydroxylase (Table I). By contrast, the loss of STAT5a did not increase expression of any of the female-dominant CYP enzyme activities assayed (testosterone 2α- and 6α-hydroxylase) in affected males (Fig. 3*A*; Table I). None of the female-dominant testosterone hydroxylase activities in female mouse liver was affected by *Stat5a* or *Stat5b* gene disruption (Table I). We conclude that the loss of STAT5a in male mouse liver does not lead to the increase in female-dominant CYP enzyme activities that occurs in response to the loss of STAT5b.

A similar conclusion can be drawn based on the effects of *Stat5a* and *Stat5b* gene disruption on sex-dependent CYP3A and CYP2B protein expression. Western blot analysis indicated that the female-dominant liver CYP3A protein *band b* was increased in *Stat5b*$^{-/-}$ males (Fig. 4*B*, *lanes 5–7 versus lanes 1–4*), in agreement with the increase in CYP3A-diagnostic testosterone 6β-hydroxylase activity (Fig. 3*B*). Two other liver CYP proteins that are female-dominant in 129J × BALB/c mice, CYP2B *band a* and CYP2B *band b*, were also increased in *Stat5b*$^{-/-}$ males to the much higher female levels (Fig. 5*A*, *lanes 5–8 versus lanes 1–4*; *cf. lane 9*). By contrast, none of the female-dominant CYP proteins, *i.e.* CYP3A *band b* and CYP2B *band b*, was increased in *Stat5a*$^{-/-}$ male mice (Fig. 4*A*, *lanes 4–7 versus lanes 1–3*; Fig. 5*B*, *lanes 4–7 versus lanes 1–3*).[3] *Stat5b*$^{-/-}$ male mice also exhibited a significant (albeit partial) loss of male-specific testosterone 16α-hydroxylase activity (Fig. 3*B*) and its associated CYP2D *band b* (Fig. 6*B*, *lanes 5–8 versus lanes 1–4*). In contrast, expression of the male-specific liver CYP2D *band b* was unchanged in *Stat5a*$^{-/-}$ male mice (Fig. 6*A*, *lanes 4–7 versus lanes 1–3*) (Table II).

We conclude that *Stat5b* gene disruption feminizes the livers of male mice by increasing the expression of female-dominant CYP3A and CYP2B proteins to the level of wild-type female mice, while partially decreasing the expression of male-domi-

[3] CYP2B *band a* is expressed in both males and females in 129J × Black Swiss mice, in contrast to its female-specific expression pattern in 129J × BALB/c mice (Fig. 5, *B versus A*).

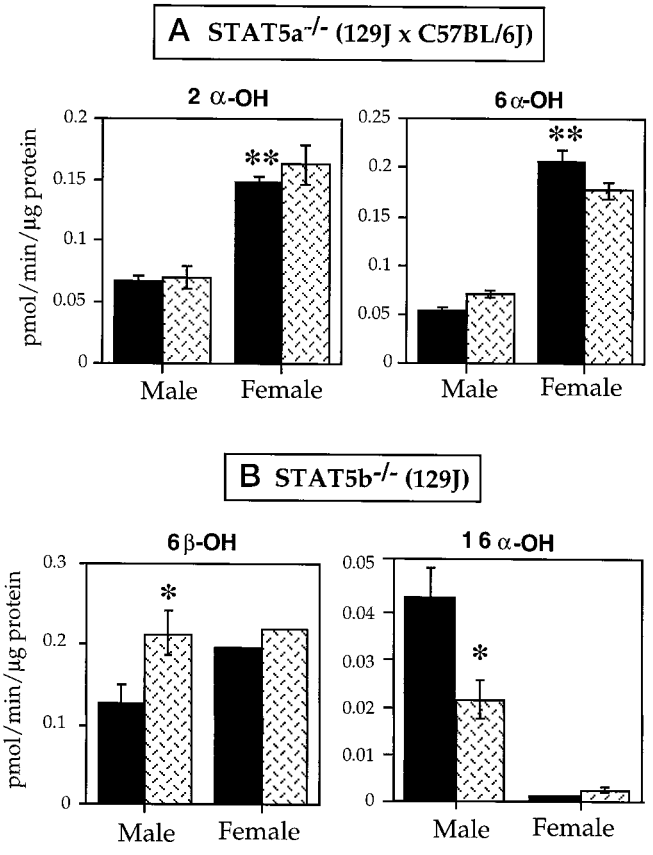

FIG. 3. **Influence of *Stat5a* and *Stat5b* gene disruption on sex-dependent liver microsomal testosterone hydroxylase activities.** Testosterone hydroxylase activities were determined in liver microsomes prepared from wild-type mice (*filled bars*) and *Stat5a*$^{-/-}$ or *Stat5b*$^{-/-}$ mice (*speckled bars*). Hepatic microsomal proteins (25 μg) were incubated with ^{14}C-labeled testosterone in the presence of NADPH as described under "Materials and Methods." Specific activities shown are mean ± S.E. for $n = 5$ individual mice per group. A *single asterisk* designates significant differences from wild-type at $p \leq 0.05$. The *double asterisks* represent significant differences from males at $p \leq 0.05$.

TABLE I
Impact of Stat5a and Stat5b gene disruption on sex-dependent liver microsomal testosterone hydroxylase activities

Hydroxytestosterone metabolite	Male		Female	
	Wild type	Knockout	Wild type	Knockout
Stat5a$^{-/-}$ (129J × Black Swiss)				
2α-OH	+	+	+++	+++
6α-OH	+	+	+++	+++
7α-OH	+	++[a]	+++	+++
Stat5b$^{-/-}$ (129J)				
6β-OH	+	+++	+++	+++
16α-OH	+++	+	−	−
Stat5b$^{-/-}$ (129J × BALB/c)				
6α-OH	+	+++	+++	+++
6β-OH	+	+++	+++	+++

[a] Partial elevation in enzyme activity was seen in some individual mice but did not reach statistical significance ($p \leq 0.15$ compared to wild-type males).

nant CYP2D9 protein and its associated testosterone 16α-hydroxylase activity to the lower level characteristic of females. These data support our earlier proposal that STAT5b is essential for maintaining the overall pattern of male-specific liver CYP gene expression (23). By contrast, STAT5a is apparently not required to maintain a male-specific pattern of GH-regulated liver CYP protein expression in male mice. Together, these studies indicate that STAT5b-STAT5b homodimers are likely to be required, while STAT5b-STAT5a heterodimers are dispensable for the male-specific pattern of liver CYP expression.

Selective Loss in Female Mice of Select CYP Enzyme Activities upon Disruption of Stat5a or Stat5b—We next investigated whether either of the STAT5 proteins is required to maintain normal CYP enzyme and protein expression in female mouse liver. Although disruption of the *Stat5a* or *Stat5b* genes had no effect in males on any of the sex-independent CYP activities examined (Fig. 7; Table III), in females the loss of STAT5a or STAT5b did result in a marked loss of some, but not all, sex-independent testosterone hydroxylase activities. Thus, liver microsomal testosterone 16α-hydroxylation was decreased in both female *Stat5a*$^{-/-}$ mice and in female *Stat5b*$^{-/-}$ mice (129J × BALB/c), while testosterone 6α-hydroxylation was decreased in *Stat5b*$^{-/-}$ females (129J strain) (Fig. 7). Other sex-independent testosterone hydroxylases were unaffected (*e.g.* 6β-hydroxylase and 2α-hydroxylase; *left panels* of Fig. 7). Although testosterone 16α-hydroxylase is a male-specific enzyme activity in 129J inbred mice (Fig. 3*B*), this activity is high in both females and males in wild-type 129J outcrossed mice (*filled bars*, Fig. 7). This latter finding is consistent with the characterization of the female mouse liver testosterone 16α-hydroxylase enzyme as a female-dominant P450 whose repression in 129J females is inherited as an autosomal recessive trait (4). Moreover, the selective loss of testosterone 16α-hydroxylase activity in female mouse liver in response to the loss of either STAT5a or STAT5b (Table III, last column; Fig. 7) indicates that both STAT5 forms are required to maintain full expression of this constitutively expressed CYP gene product in female but not male mice. Similarly, the selective loss in female 129J *Stat5b*$^{-/-}$ mouse liver of testosterone 6α-hydroxylase activity (Fig. 7*B*, *right panel*) indicates a requirement for both STAT5 proteins to maintain expression of this P450 enzyme in females.

Loss of Female-specific CYP2B Protein in Female Stat5a$^{-/-}$ *Mice*—The mouse CYP2B subfamily contains several liver-expressed proteins, including at least one whose expression is female-predominant and growth hormone-regulated (6). In view of the GH dependence of this CYP2B enzyme, we examined whether *Stat5a* or *Stat5b* disruption impacts on its expression. Western blot analysis revealed three CYP2B cross-reactive proteins in 129J × Black Swiss mouse liver microsomes: *band a*, which is sex-independent in this mouse strain,[3] albeit variable in individual mice (Fig. 5*B*); *band b*, which is female-specific; and *band c*, which in some experiments could be resolved to give two bands (Figs. 5, *A* and *C*). In male mice, *Stat5a* gene disruption did not cause any notable change in the level of the sex-independent CYP2B *band a* or any increase in the female-specific CYP2B *band b* (Fig. 5*B*), as indicated above. By contrast, a substantial loss of the female-specific *band b* was seen in five of six female *Stat5a*$^{-/-}$ mice (Fig. 5*C*, *lanes 7–11 versus lanes 2–6*, Table II, and data not shown). This response of CYP2B *band b* to *Stat5a* gene disruption in females is in sharp contrast to the lack of an effect of *Stat5a* gene disruption on other female-specific or female-dominant liver CYP proteins or activities, such as CYP3A *band b* (Fig. 4*A*) or testosterone 2α- and 6α-hydroxylase activity (Fig. 3*A*). CYP2B *band b* was not expressed in 129J male or female mouse liver, precluding a determination of the impact of *Stat5b* gene disruption on its expression in this strain. In 129J × BALB/c outbred mice, CYP2B *bands a* and *band b* were both expressed as female-dominant forms (Fig. 5*A*); *band a* remained at the same level of expression in female *Stat5b*$^{-/-}$ mice, while *band b* was partially decreased in five of seven female *Stat5b*$^{-/-}$ mice (data not shown).

Relative Expression Levels of STAT5a and STAT5b mRNA in

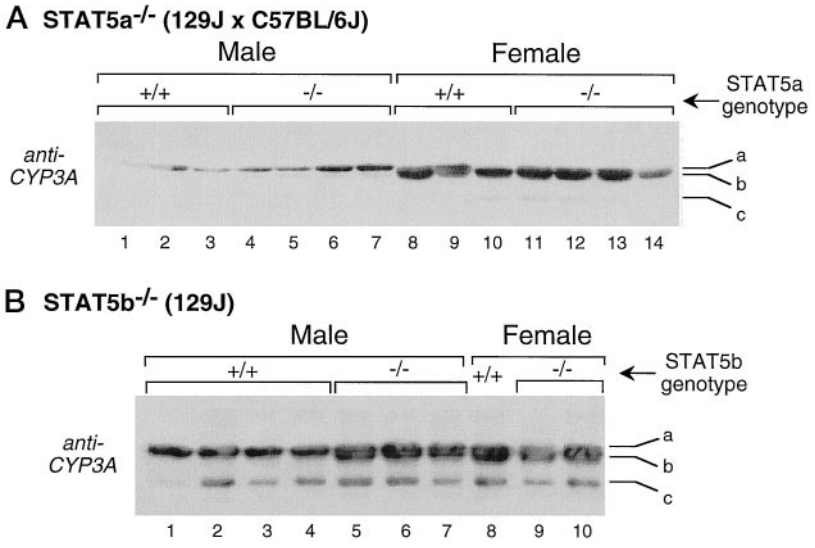

FIG. 4. **CYP3A protein expression in *Stat5a*$^{-/-}$ and *Stat5b*$^{-/-}$ mice.** Shown are Western blots of mouse liver microsomes (20 μg) prepared from wild-type and *Stat5a*$^{-/-}$ (*A*) and *Stat5b*$^{-/-}$ mice (*B*) using anti-CYP3A antibodies. Of the three immune cross-reactive CYP3A bands, the female-dominant *band b* was significantly elevated in *Stat5b*$^{-/-}$ male mice (*B*, *lanes 5–7*). No significant changes were observed in either male or female *Stat5a*$^{-/-}$ mouse liver microsomes. *Band b* is somewhat difficult to distinguish from *band a* in *panel B*, in part due to the fuzzy nature of the bands seen on this Western blot.

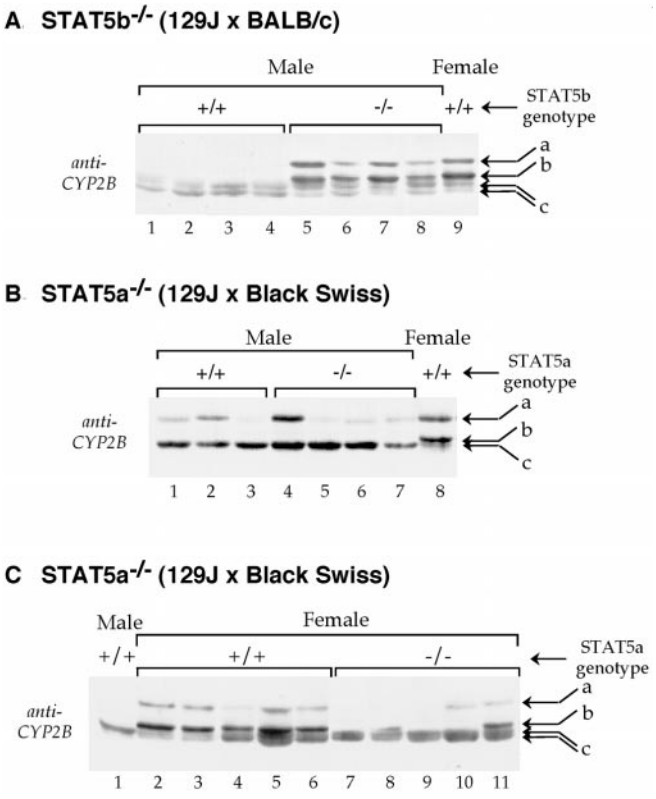

FIG. 5. **Effect of *Stat5a* gene disruption on hepatic microsomal CYP2B protein expression.** Shown are Western blots of male and female mouse liver microsomes from the indicated strains, probed using anti-CYP2B polyclonal antibody. Three immune cross-reactive CYP2B bands are seen (*bands a*, *b*, and *c*, with *band c* resolved into a doublet in *A* and *C*). CYP2B *band a* is female-specific in wild-type 129 × BALB/c mice (*A*) but is expressed in both sexes in 129J × Black Swiss mice (*B*). CYP2B *band b* is female-specific in both strains.

Mouse Liver—In view of the potential role of STAT5 heterodimerization in regulating CYP enzyme expression suggested by the above experiments, we sought to determine the relative abundance of STAT5a and STAT5b in mouse liver. STAT5 form-specific antibodies are available (*e.g.* Fig. 1); however, the absence of purified STAT5 protein standards precluded a determination of the relative molar abundance of STAT5a *versus* STAT5b protein using an immunochemical approach. Consequently, we investigated the relative levels of STAT5a and STAT5b mRNA in total mouse liver RNA using a reverse transcription PCR method. Since the two STAT5 mRNAs are ~90% identical, we used an assay that incorporates an *Nco*I restriction digestion step to distinguish a PCR-amplified STAT5a cDNA fragment, which contains an *Nco*I site, from the corresponding STAT5b cDNA, which does not (see "Materials and Methods"). Results obtained from five individual 129J × Black Swiss mouse livers are shown in Fig. 8*A*. *Nco*I digestion of the PCR fragments revealed that the majority of the STAT5 cDNA was derived from STAT5b mRNA in both male (Fig. 8*A*) and female 129J × Black Swiss mice (data not shown), as indicated by the minor fraction that was digested to yield the STAT5a-derived 310- and 240-bp *Nco*I fragments. In control experiments, *Nco*I fully digested STAT5a cDNA amplified from a cloned STAT5a plasmid using the same PCR primers, while there was no digestion of a corresponding STAT5b cDNA fragment (data not shown). Quantitation of the ratio of digested to undigested fragments revealed that STAT5b corresponded to 95–96% of the total STAT5 mRNA in both male and female 129J and 129J × Black Swiss mice and to ~90% of the total STAT5 mRNA in 129J × BALB/c mice (Fig. 8*B* and data not shown).

DISCUSSION

The present study demonstrates that STAT5a and STAT5b both play important roles in the maintenance of sexually dimorphic liver CYP gene expression in the mouse model. The two highly conserved (~90% identical) STAT5 proteins, STAT5a and STAT5b, were shown to be activated in mouse liver to form both homodimers (STAT5a-STAT5a; STAT5b-STAT5b) and heterodimers (STAT5a-STAT5b). STAT5b alone, most likely in the form of a STAT5b-STAT5b homodimeric complex, was found to be required to maintain the male-specific pattern of GH pulse-stimulated liver *Cyp* expression. This was apparent from the loss of the male-specific CYP2D9 *band b* and the increase in several female-predominant P450s in *Stat5b*$^{-/-}$ but not *Stat5a*$^{-/-}$ male mouse liver (CYP3A *band b* and CYP2B *band b)*. By contrast, in female mouse liver, STAT5a and STAT5b were both required for full expression of several CYP proteins and activities, including a female-specific CYP2B protein (*band b*; Fig. 5*C*), which apparently corresponds to the female-specific, GH-regulated mouse liver CYP2B protein with the same relative mobility described elsewhere (6). This requirement of both STAT5a and STAT5b in female mouse liver suggests that STAT5a-STAT5b heterodimers play a unique regulatory role in the female. This contrasts with the role proposed for STAT5b homodimers in male liver in mediating

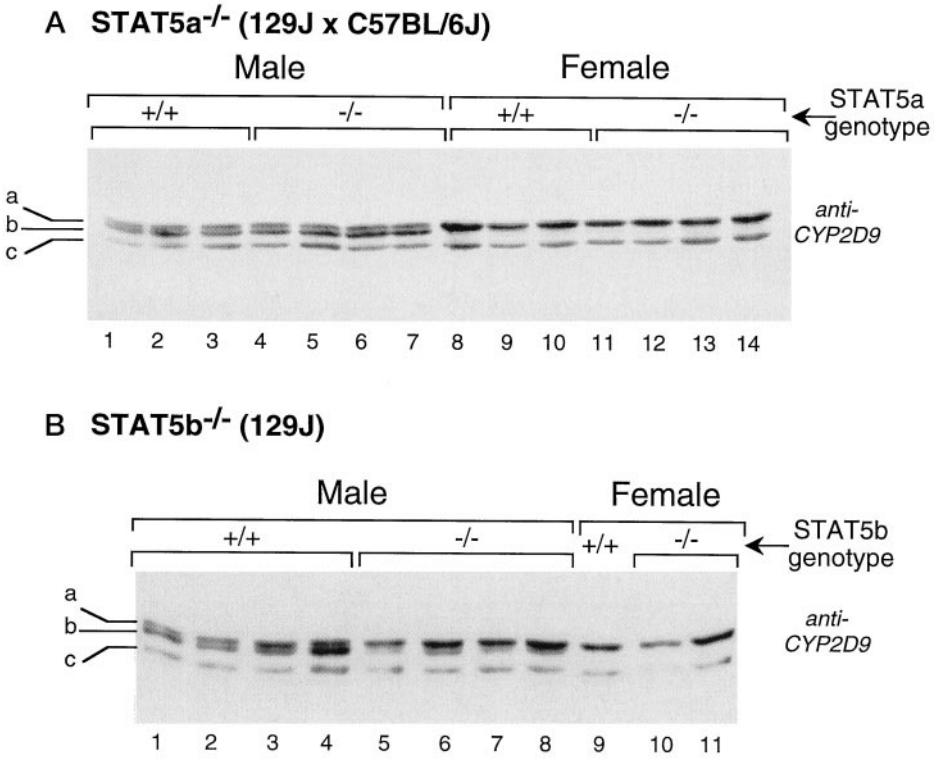

FIG. 6. **Effect of *Stat5b*$^{-/-}$ on male-specific CYP2D9 protein expression in 129J mice.** Shown are Western blots of mouse liver microsomes probed using anti-mouse CYP2D9 antibody. *Band b* shows the male predominance that is characteristic of CYP2D9. This band is decreased near to female levels in male *Stat5b*$^{-/-}$ mice but is unchanged in male *Stat5a*$^{-/-}$ mice.

TABLE II
Impact of Stat5a and Stat5b gene disruption on sex-dependent P450 protein levels

Expression of the indicated hepatic microsomal CYP proteins was determined by Western blotting (*e.g.* as shown in Figs. 4–6).

	Male	Female
CYP3A, *band b* (female-specific)		
Wild type	–	+++
Stat5a$^{-/-}$ (129J × Black Swiss)	–	+++
Stat5b$^{-/-}$ (129J)	+++	+++
Stat5b$^{-/-}$ (129J × BALB/c)	+++	+++
CYP2B, *band b* (female-specific)		
Wild type	–	+++
Stat5a$^{-/-}$ (129J × Black Swiss)	–	–
Stat5b$^{-/-}$ (129J × BALB/c)	+++	+++
CYP2D9, *band b* (male-dominant)		
Wild type	+++	–
Stat5a$^{-/-}$ (129J × Black Swiss)	+++	–
Stat5b$^{-/-}$ (129J)	+	–
Stat5b$^{-/-}$ (129J × BALB/c)	–	–

GH pulse regulation of sexually dimorphic liver CYPs. Although a definitive cause-and-effect relationship is not established by these findings, the loss of male-specific liver gene expression in *Stat5b*$^{-/-}$ but not *Stat5a*$^{-/-}$ mice could indicate a direct effect of, and a specific requirement for, STAT5b-STAT5b homodimers to regulate expression of male GH pulse-induced *Cyp* genes. Alternatively, it is possible that other STAT5-containing complexes (STAT5a-STAT5b and STAT5a-STAT5a) could be intrinsically capable of regulating the male-expressed *Cyp* genes but might simply not be present in sufficient amounts in *Stat5b*$^{-/-}$ mouse liver to satisfy the threshold requirements with respect to transmission to the nucleus of a male, pulsatile plasma GH signal.

Strain-dependent Expression of Mouse Liver CYP Testosterone Hydroxylases—In contrast to the rat, where at least 24 hepatic CYP forms have been extensively characterized at a molecular and regulatory level and CYP gene-specific catalytic and immunochemical probes are widely available (39), the characterization of individual murine members of the CYP enzyme system is far less advanced, and in many instances the relationship of specific mouse P450 proteins with specific *Cyp* genes is uncertain. Moreover, unlike in the rat, there are major strain differences in *Cyp* gene expression patterns in the mouse (4, 34, 35) (Tables I and III). For example, whereas testosterone 15α-hydroxylase activity has been associated with the female-predominant CYP2A4, and testosterone 16α-hydroxylase activity represents the male-specific CYP2D9 in some mouse strains, these P450 enzyme activities are also associated with other CYP gene products and consequently lose their sex-dependence in other mouse strains (Table III) (4, 31). For example, testosterone 16α-hydroxylation is not only catalyzed by CYP2D9, but is also catalyzed by a female-expressed CYP2B enzyme that is expressed in select mouse strains (4, 5, 40, 41). Accordingly, the high level of liver microsomal testosterone 16α-hydroxylase activity seen in both male and female wild-type 129J × Black Swiss and 129J × BALB/c mice (*versus* the male-specific expression of testosterone 16α-hydroxylase activity in 129J mice) (Fig. 7, *A* and *C*, *versus* Fig. 3*B*) probably results from the combined expression in the outcrossed strains of the male-specific CYP2D9 *band b* (*e.g.* Fig. 6) and the female-specific CYP2B, *band b* (Fig. 5). CYP2B *band b* is not expressed in 129J mice (data not shown), in agreement with the repression via an autosomal recessive trait of the female-predominant testosterone 16α-hydroxylase CYP enzyme in 129J females (4). Consequently, the selective loss in female, but not male, STAT5a and STAT5b knockout mice of certain sex-independent testosterone hydroxylase activities, namely 16α-hydroxylation (129J × BALB/c and 129J × Black Swiss) and 6α-hydroxylation (129J), is likely to reflect a loss of female-specific CYP gene products rather than a loss in the female of sex-independent CYP gene products. Further progress in linking the individual mouse liver microsomal testosterone hydroxylase activities of each strain with their corresponding *Cyp* genes will be necessary in order to further investigate at the gene-regulatory level the differential effects of STAT5a-STAT5b heterodimers *versus* STAT5b-STAT5b homodimers on liver CYP gene expression that we hypothesize to occur on the

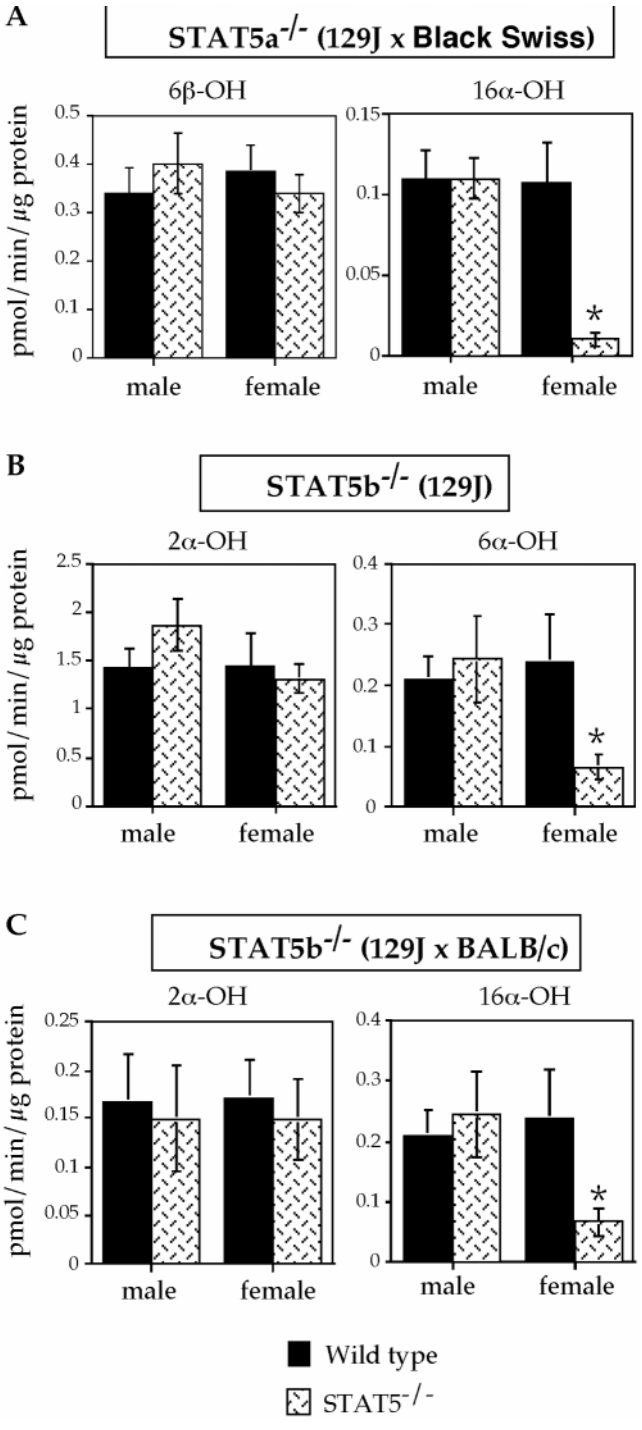

FIG. 7. **Effect of *Stat5a*$^{-/-}$ or *Stat5b*$^{-/-}$ on sex-independent liver testosterone hydroxylase activities.** Testosterone hydroxylase activities were determined in liver microsomes prepared from wild-type mice (*filled bars*) and *Stat5a*$^{-/-}$ or *Stat5b*$^{-/-}$ mice (*speckled bars*) as described under "Materials and Methods." Specific activities shown are mean ± S.E. for $n = 5$ individual mice per group. A *single asterisk* represents significant differences from wild type ($p \leq 0.05$). Disruption of *Stat5a* or *Stat5b* is seen to decrease expression in female but not male mouse liver of some but not other sex-dependent, CYP-catalyzed testosterone hydroxylase activities.

TABLE III
Effect of Stat5a and Stat5b gene disruption on sex-independent liver microsomal CYP-catalyzed testosterone hydroxylase activities

Hydroxytestosterone Metabolite	Male	Female
Stat5a$^{-/-}$ (129J × Black Swiss)		
6β-OH	NC[a]	NC
15α-OH	NC	NC
16β-OH	NC	NC
16α-OH	NC	↓ ↓ ↓ [b]
Stat5b$^{-/-}$ (129J)		
2α-OH	NC	NC
15α-OH	NC	NC
16β-OH	NC	NC
6α-OH	NC	↓ ↓ ↓
Stat5b$^{-/-}$ (129J × BALB/c)		
2α-OH	NC	NC
7α-OH	NC	NC
15α-OH	NC	NC
16α-OH	NC	↓ ↓ ↓

[a] NC, no change in enzyme level in response to either *Stat5a* or *Stat5b* gene disruption.
[b] ↓ ↓ ↓, the indicated testosterone hydroxylase activities were decreased only in females.

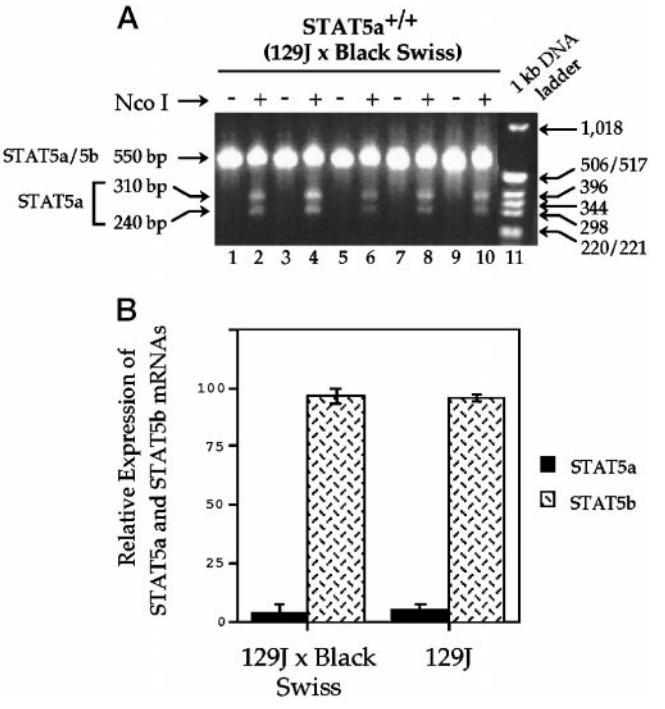

FIG. 8. **Reverse transcription PCR analysis of liver STAT5 mRNA levels.** 2 μg of total RNA isolated from five individual male 129J × Black Swiss mouse livers was reverse-transcribed and then PCR-amplified with STAT5 primers that do not distinguish between STAT5a and STAT5b, as described under "Materials and Methods." *A*, the amplified PCR products (550 bp), with or without *Nco*I digestion, as indicated, were electrophoresed in an ethidium bromide-stained gel. The 550-bp STAT5b cDNA fragment does not contain an *Nco*I site, whereas the STAT5a cDNA is digested by *Nco*I to yield fragments of 240 and 310 bp. *B*, quantitation of the fractions of total STAT5 cDNA that correspond to STAT5a (310- plus 240-bp fragments) and STAT5b (550-bp fragment) after *Nco*I digestion, as determined by integration of band intensities. In control experiments not shown, the corresponding 550-bp cDNA amplified from cloned STAT5 plasmid DNA was shown to be fully digested with *Nco*I in the case of STAT5a and not at all digested with *Nco*I in the case of STAT5b.

basis of our findings. Identification and further investigation of the specific *Cyp* genes that may be regulated in this manner is likely to be a formidable task, in view of the complexity of the mouse *Cyp* gene superfamily (*cf.* three distinct classes comprised of 16 closely related mouse *Cyp2b* genes, only two of which have been identified (42)).

Proposed Role of Hormone-induced Activation and Dimerization of STAT5a and STAT5b—In mammary gland, STAT5a and STAT5b are both activated by prolactin (24). The generation of mice in which the genes encoding prolactin receptor (43), STAT5a (25), and STAT5b (23) have been individually inactivated has demonstrated that in mammary gland the het-

erodimeric STAT5a-STAT5b complex is the principal mediator of mammopoietic and lactogenic signaling (44). Since STAT5a and STAT5b can thus be activated by prolactin, in addition to GH, the decreased expression of certain *Cyp* genes in both *Stat5a*$^{-/-}$ and *Stat5b*$^{-/-}$ female mouse liver described in the present study could conceivably reflect a loss of STAT5a- and STAT5b-mediated liver prolactin signaling. However, while the long form of prolactin receptor is expressed in liver tissue, the short form of the receptor is much more abundant. Moreover, prolactin receptor short form can exert a dominant-negative phenotype with respect to STAT activation, such that prolactin-dependent STAT5 activation is not achieved in liver tissue (12, 45, 46). Accordingly, female *Cyp* gene expression in the liver is not likely to be regulated by prolactin-induced STAT5a-STAT5b activation and heterodimerization, but rather by GH or perhaps other cytokines that can also activate STAT5a and STAT5b (47–49). The factors that regulate the extent of dimerization between STAT5a and STAT5b have not been identified, although the relative abundance of activated STAT5a and STAT5b in the target tissue is likely to be a key factor. In liver tissue, STAT5a appears to be the more minor expressed STAT5 form, both in the rat (36, 37) and in the mouse (Fig. 8). Accordingly, the ratio of activated STAT5b to STAT5a may be much greater than 1, such that homodimeric STAT5b complexes dominate, particularly in males, where the pulsatile plasma GH profile activates STAT5b much more efficiently than in females (12). It is conceivable, however, that in female liver activated heterodimeric STAT5a-STAT5b complexes may be relatively abundant as a consequence of the down-regulation of STAT5b activation in response to the female-characteristic, nearly continuous plasma GH profile (12, 33, 50). Thus, whereas the abundance of activated, homodimeric STAT5b complexes may serve to maintain sexually dimorphic GH responses in male liver, a heterodimeric STAT5a-STAT5b complex in female liver could contribute to the expression of certain female-specific *Cyp* genes, such as that which encodes CYP2B *band b*.

Implications for GH-regulated CYP Gene Expression—In males, but not females, *Stat5b* gene disruption increased female-dominant testosterone hydroxylase enzyme activity and protein expression (Tables I and II). This elevation of normally female-dominant liver enzyme levels suggests that STAT5b may negatively regulate some GH-regulated liver-expressed genes in addition to its demonstrated positive effects on the transcriptional activation of certain male GH pulse-stimulated genes (22).[2] This model is in accord with other studies based on an analysis of CYP enzyme patterns in GH-deficient *lit/lit* mice, where it is shown that the low expression in male mouse liver of several female-specific, GH-regulated CYP enzymes is at least in part due to the suppressive effects of male GH pulses (2, 6). Precedent for an inhibitory effect of activated STAT5b on gene expression is provided by the transcriptional inhibition by prolactin-activated STAT5b of interferon-regulatory factor-1 (51) and by the inhibitory effects of GH-activated STAT5b on gene transcription stimulated by cross-talk with the nuclear receptor PPARα (52). Thus, while GH pulse-induced expression of male-specific *Cyp* genes such as rat *CYP2C11* or mouse *Cyp2d9* may require direct STAT5b-DNA interactions, we hypothesize that GH-dependent *Cyp* gene products that are female-expressed (*e.g.* rat *CYP2C12* or mouse CYP2B, *band b*) may in part be regulated by interactions between STAT5b and other factors (*e.g.* a hypothetical repressor of the female-expressed *Cyp* genes). These interactions could lead to inhibition of gene expression in the male, giving rise to the observed female-specific pattern of gene expression.

Distinct intracellular signaling pathways are activated by a nearly continuous (female) compared with an intermittent (male) pattern of plasma GH stimulation and have been implicated in the sex-dependent expression of certain *Cyp* genes in rat liver (1). These include the JAK/STAT5b pathway in the case of males (12) and pathway(s) that may involve a novel GH-regulated nuclear factor, termed GHNF (53) and perhaps also phospholipase A2 signaling (54) in females. In addition, the present study demonstrates that in female mouse liver STAT5a and STAT5b are both required for expression and thus may be important regulators, of certain female-specific CYP enzymes. The interactions of STAT5 with female-expressed *Cyp* genes may be direct, *e.g.* could involve STAT5a-STAT5b DNA-protein complexes, or may be indirect. Conceivably, *Cyp* genes, even within the same gene subfamily, may respond to distinct GH-dependent signaling pathways. Alternatively, a single GH-activated signaling pathway may regulate different *Cyp* genes, in some cases leading to activation and in other cases leading to inhibition of *Cyp* expression. Further study will be required to elucidate the multiple GH-activated signaling pathways that activate members of this multigene family and to establish the precise roles that STAT5a and STAT5b play in their expression.

After completion of the present study, Teglund *et al.* (55) confirmed our earlier report (23) that *Stat5b* gene disruption leads to loss of the GH pulse-regulated male pattern of postpubertal body growth rate and liver gene expression. They also reported the lack of an effect of *Stat5a* gene disruption on male-specific CYP2D expression, in agreement with the present study.

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