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

Molecular Endocrinology 20(6):1333–1351

doi: 10.1210/me.2005-0489

Sex-Dependent Liver Gene Expression Is Extensive and Largely Dependent upon Signal Transducer and Activator of Transcription 5b (STAT5b): STAT5b-Dependent Activation of Male Genes and Repression of Female Genes Revealed by Microarray Analysis

Karl H. Clodfelter, Minita G. Holloway, Paul Hodor, Soo-Hee Park, William J. Ray, and David J. Waxman

Division of Cell and Molecular Biology (K.H.C., M.G.H., S.-H.P., D.J.W.), Department of Biology, Boston University, Boston, Massachusetts 02215; and Department of Molecular Endocrinology (P.H., W.J.R.), Merck Research Laboratories, West Point, Pennsylvania 19486

Sexual dimorphism in mammalian liver contributes to sex differences in physiology, homeostasis, and steroid and foreign compound metabolism. Many sex-dependent liver genes are regulated by sex differences in pituitary GH secretion, with the transcription factor, signal transducer and activator of transcription (STAT5b), proposed to mediate signaling by the pulsatile, male plasma GH profile. Presently, a large-scale gene expression study was conducted using male and female mice, wild type and Stat5b inactivated, to characterize sex differences in liver gene expression and their dependence on STAT5b. The relative abundance of individual liver RNAs was determined for each sex-genotype combination by competitive hybridization to 23,574-feature oligonucleotide microarrays. Significant sex differences in hepatic expression were seen for 1603 mouse genes. Of 850 genes showing higher expression in males, 767 (90%) were down-regulated in STAT5b-deficient males. Moreover, of 753 genes showing female-predominant expression, 461 (61%) were up-regulated in STAT5b-deficient males. In contrast, approximately 90% of the sex-dependent genes were unaffected by STAT5b deficiency in females. Thus: 1) STAT5b is essential for sex-dependent liver gene expression, a characteristic of approximately 1600 mouse genes (4% of the genome); 2) male-predominant liver gene expression requires STAT5b, or STAT5b-dependent factors, which act in a positive manner; and 3) many female-predominant liver genes are repressed in males in a STAT5b-dependent manner. Several of the STAT5b-dependent male genes encode transcriptional repressors; these may include direct STAT5b targets that repress female-predominant genes in male liver. Several female-predominant repressors are elevated in STAT5b-deficient males; these may contribute to the major loss of male gene expression seen in the absence of STAT5b. (*Molecular Endocrinology* 20: 1333–1351, 2006)

TRANSCRIPTION FACTORS belonging to the STAT (signal transducer and activator of transcription) family regulate the expression of genes involved in a wide range of biological processes, including embryonic development, cell growth regulation, innate and adaptive immunity, and organogenesis (1, 2). STAT5b, one of seven mammalian STAT proteins, responds to a variety of extracellular cytokine and growth factor signals (3), including interleukins, epidermal growth factor and GH (4–6). GH regulates gene expression in several tissues, most notably liver. GH is secreted by the pituitary gland in a sex-dependent manner and under the regulation of gonadal steroids (7, 8). This, in turn, leads to substantial sex differences in GH-regulated liver gene expression (9). In the rat, plasma GH levels are highly pulsatile in males, where hormone peaks approximately every 3.5 h are followed by a GH-free interval lasting about 2 h, whereas in females, GH is present in the plasma in a nearly continuous manner. Mice also show sexually dimorphic GH-secretory patterns, with females characterized by more frequent GH pulses and a shorter GH-free interpulse interval than males (10). These sex-dependent plasma GH profiles regulate liver gene expression at the level

First Published Online February 9, 2006

Abbreviations: CYP, Cytochrome P450; GO, Gene Ontology; ID, identification; JAK, Janus family of tyrosine kinases; KEGG, Kyoto Encyclopedia of Genes and Genomes; KO, knockout; MUP, major urinary protein; qPCR, quantitative PCR; STAT, signal transducer and activator of transcription; WT, wild type.

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

of transcription, as demonstrated for several GH-regulated liver cytochrome P450 (*Cyp*) genes (11, 12) (for review, see Ref. 13).

GH binding to its cell surface receptor activates JAK (Janus family of tyrosine kinases) 2, a GH receptor-associated tyrosine kinase. JAK2, in turn, phosphorylates GH receptor on multiple cytoplasmic domain tyrosine residues, several of which serve as docking sites for STAT5b (6). STAT5b is then phosphorylated on Tyr 699, which enables it to dimerize and translocate to the nucleus, where it binds to STAT5 response elements. STAT5b is directly activated in male rat liver in response to each incoming plasma GH pulse, whereas in female rats, the persistence of plasma GH stimulation leads to an apparent partial desensitization of the STAT5b signaling pathway and substantially lower nuclear STAT5b protein than the peak levels seen in males (14–17). A similar sexual dimorphism characterizes nuclear STAT5b activity in mouse liver (18). Based on these findings, STAT5b has been proposed to serve as a mediator of the sex-dependent effects that GH has on liver gene expression (19). This proposal is supported by the characterization of STAT5b-deficient male mice, which display a reduced body growth rate at puberty and a loss of sex-specific liver expression of several *Cyps* and other genes (20–22).

Microarray technology has been applied to the study of GH-regulated liver gene expression and has helped elucidate responses to hypophysectomy (23, 24) and GH replacement (23, 25, 26) and the impact of genetic models of GH deficiency (27, 28) and chronic GH treatment (29–31) on gene expression. Chronic GH treatment of rats reverses the effects of hypophysectomy on approximately 60 liver-expressed genes, in addition to about 30 genes expressed in heart and kidney (23). Moreover, continuous GH infusion in male rats imparts an overall female pattern of liver gene expression, both at the RNA level (29) and the nuclear protein level (32), evidencing the responsiveness of both male-predominant and female-predominant genes to changes in the plasma GH profile. The role of STAT5b in these effects of GH on liver gene expression, however, is not known. Given the differential responsiveness of STAT5b to the sex-dependent plasma GH profiles, noted above, it is of interest to investigate the STAT5b-knockout (KO) mouse model (22) to ascertain whether the loss of STAT5b has a global impact on liver gene expression.

The present study uses microarray technology to investigate the impact of STAT5b deficiency on liver gene expression, in particular, sex-dependent liver gene expression. Disruption of the *Stat5b* gene is shown to lead to a marked loss of sex-specific gene expression in male liver whereas it has much more modest effects in female liver. Several sex-specific transcriptional activators and repressors were identified and shown to be dependent on STAT5b for expression, suggesting that one or more of these factors may participate in a STAT5b-dependent signaling cascade that regulates sex-dependent liver genes, including *Cyp* genes. These latter findings may help elucidate the mechanisms through which STAT5b and GH regulate their many target genes in liver tissue.

RESULTS

Experimental Design

To characterize sex differences in liver gene expression and any requirement of STAT5b for maintaining these differences, a large-scale gene expression study was conducted using RNA isolated from the livers of male (M) and female (F) mice that were either wild-type (WT) or had the *Stat5b* gene inactivated (KO). Three pools of liver RNA were prepared for each of the four sex-genotype combinations (M or F, and WT or KO), and the relative abundance of individual RNAs within these pools was determined by competitive hybridization to 23,574-feature oligonucleotide microarrays. Four sets of competitive two-color hybridizations were carried out: 1) M-WT *vs.* F-WT; 2) M-WT *vs.* M-KO; 3) F-KO *vs.* F-WT; and 4) F-KO *vs.* M-KO.

Hybridization data were normalized to adjust for differences in overall signal intensity, and expression ratios were calculated and used to identify genes showing significant differences between each sex-genotype combination. Almost all of the reporters on the array (23,455 of 23,574) gave signal intensities at least 2-fold greater than background (*i.e*. signal from a spot without probe). Of these, 2267 (9.7%) had at least one of the four average expression ratios meet both a significance level of $P < 0.05$ and a 1.5-fold threshold for differential expression, indicating that these genes were expressed in a sex-specific and/or STAT5b-dependent manner. Elimination of duplicate reporters and reporters that could not be unequivocally related to a specific gene reduced the number of regulated genes to 2231. These 2231 genes were reproducibly expressed in a sex-specific manner in either WT mice or STAT5b-KO mice or were expressed in a STAT5b-dependent manner, and are listed in supplemental Table 1 published on The Endocrine Society's Journals Online web site at http://mend.endojournals.org.

Sex-Specific Gene Expression in WT Mice

A majority of the differentially expressed genes, 1603 (72%) of 2231 genes, showed differential expression between WT males and WT females and are thus defined as sexually dimorphic. These genes are colored *green* (M-WT $>$ F-WT) or *red* (M-WT $<$ F-WT) in Fig. 1A, lane 1, where they are displayed at the *far ends* of a false-color heat map containing all 2231 genes sorted by average M-WT:F-WT ratio. Whereas several of these genes were previously identified as sex-dependent, including many members of the *Cyp* superfamily (see below), most are novel observations. Expression of 850 of the 1603 genes was male predominant in WT mouse liver (M-WT:F-WT $\geq$ 1.5; first

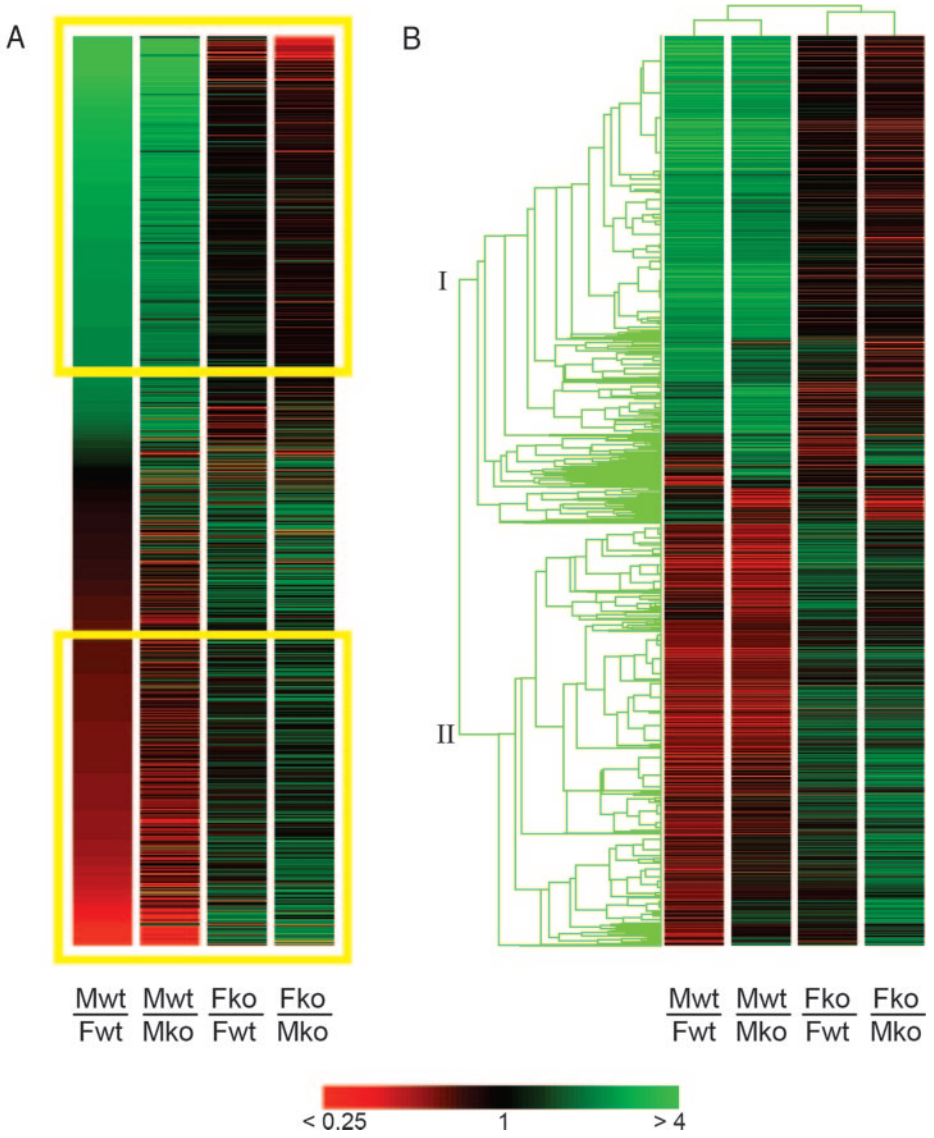

Fig. 1. False Color Heat Maps and Hierarchical Clustering for Expression Profiles of 2231 Differentially Expressed Genes across Four Microarrays

Genes are depicted based on their average expression ratios across four experimental pairings: M-WT:F-WT, M-WT:M-KO, F-KO:F-WT, and F-KO:M-KO. Genes are colored according to the *color bar at the bottom*, ranging from *bright green* for an average expression ratio greater than 4 to *bright red* for an average expression ratio less than 0.25, with *black* corresponding to a ratio of 1. A, Genes are sorted according to the average M-WT:F-WT ratio. The *upper and lower yellow boxes* enclose genes determined to be male specific and female specific, respectively (M-WT:F-WT >1.5 or <0.66 at $P < 0.05$). B, Genes are listed according to the hierarchical clustering method described in *Materials and Methods*. The *left dendogram* is constructed based on the Pearson's correlation values for the profiles of the four average ratios per gene. The *top dendogram* is constructed based on the Pearson's correlation of the profiles for the four separate paradigms. The length of the branches is based on the similarity distance (1 − Pearson's correlation coefficient) between the leaves joined into a new cluster. The *top-level branches* on the *left dendogram*, I and II, indicate the two most dissimilar clusters, which are split primarily upon sex specificity in the WT. The most similar experimental ratios across all 2231 genes, shown by the *shortest branches in the top dendogram*, were M-WT:F-WT and M-WT:M-KO.

column, *upper yellow box*, Fig. 1A). The remaining 753 genes were female predominant (M-WT:F-WT ≤ 1.5; first column, *lower yellow box*, Fig. 1A).

Overview of the Impact of STAT5b Deficiency

Further analysis revealed that a large majority of the male-predominant genes (Fig. 1A; genes colored *green* in lane 1) were down-regulated in STAT5b-deficient males (genes colored *green* in lane 2), whereas very few of the female-predominant genes were down-regulated in the absence of STAT5b. In fact, a significant fraction of the female-predominant genes were up-regulated in the STAT5b-deficient males (Fig. 1A, lane 2; genes colored *red* in *lower*

yellow box). Some of the female-predominant genes were also up-regulated in the STAT5b-deficient females (Fig. 1A, lane 3; genes colored *green* in *lower yellow box*). Similar patterns were observed when the genes were clustered by similarity of expression ratio and displayed in a false color heat map (Fig. 1B). The expression profiles were also clustered by Pearson's correlation coefficient, as represented by the tree to the *left* of the heat map. The primary division of the tree, between branch I and branch II, indicates that sexual dimorphism in the WT is the greatest discriminator in the four sets of expression profiles. The response of male-predominant genes across the four experimental conditions was opposite to the response of female-predominant genes, as indicated by the highly significant negative Pearson's correlation coefficient between the two major branches (Pearson = −0.999). When the same hierarchical clustering method was applied to the four sets of microarray data, as shown by the tree *above* the heat map, the M-WT:F-WT expression ratios clustered with the M-WT:M-KO expression ratios with a high correlation coefficient (Pearson = 0.861). Thus, sex specificity in the WT and response to the loss of STAT5b in males have common expression patterns.

Clustering by Significance and Differential Expression

The general trends in expression, summarized above, were further investigated by classification of the 2231 regulated genes into subgroups using a system of flags assigned to each gene on the basis of the expression ratios observed in each of the four hybridization experiments (see *Materials and Methods*). The six groups with the largest number of genes are presented in Tables 1, 2, and 3, where the 25 genes with the largest changes in M-WT:F-WT expression ratios are listed for each pair of groups. Approximately 96% of the 2231 genes were distributed among 22 of the 63 possible gene groups (Table 4). The largest group, containing 560 genes (group 1A), is comprised of male-predominant genes that were down-regulated in STAT5b-deficient male liver but showed no significant response to STAT5b deficiency in female liver and no sex specificity in the STAT5b-KO strain (Table 1A). The next two largest male-predominant groups (groups 2A and 3A) showed down-regulation in STAT5b-deficient males with partial retention of sex specificity in the STAT5b-KO strain (Tables 2A and 3A). Group 2A genes, but not group 3A genes, also showed down-regulation in STAT5b-deficient females. Three of the five largest female-predominant groups (groups 1B, 2B, and 3B) all showed up-regulation in STAT5b-deficient male liver (Tables 1–3) but differed from each other in the effect of STAT5b deficiency on gene expression in female liver (no effect for groups 1B and 3B; increased expression for group 2B) and whether sex specificity was at least partially retained in the STAT5b-KO strain (groups 2B and 3B only) (Table 4).

In the case of certain multigene families, individual members of the gene family displayed the same sex dependence and the same response to STAT5b deficiency and thus reside within the same group. The mouse major urinary protein (*Mup*) family (33–35) is one example of how this classification scheme enriches the data to isolate individual gene families. Thus, all six regulated *Mup* family and *Mup* family-related genes cosegregate in group 2A, insofar as they are male-predominant genes and were down-regulated in both male and female STAT5b-KO liver, with retention of at least partial sex specificity in the STAT5b-KO strain (Table 5A). Similarly, nine of 11 UDP-glucuronosyl transferase enzymes were found to be male predominant and down-regulated in the absence of STAT5b, with six of those members belonging to group 1A (see supplemental Table 11 published on The Endocrine Society's Journals Online web site at http://mend.endojournals.org). In contrast, although many of the *Cyp* genes were sex-dependent and responded to the loss of STAT5b in male liver, they fell into several different regulatory classes when grouped based on the impact of STAT5b deficiency in female liver (Table 5B). Several other gene families and related genes were isolated from the full set of 2231 regulated genes by term-searching the text descriptors and annotations of each gene, as summarized in supplemental Table 2 published on The Endocrine Society's Journals Online web site at http://mend.endojournals.org. Detailed listings for each of these families are presented in supplemental Tables 3–16.

Sex-Specific Response to the Loss of STAT5b

Venn diagrams were used to highlight the impact of STAT5b deficiency on the expression patterns of the 1603 sexually dimorphic genes, based on the three STAT5b KO ratios (M-WT:M-KO, F-KO:F-WT, and F-KO:M-KO) (Fig. 2). Ninety percent of the male-predominant genes (768/850) showed differential expression in response to the loss of STAT5b in male liver (M-WT:M-KO ratio; Fig. 2A). All except one of the 768 genes were expressed at a lower level in the STAT5b-KO males than in WT males (Table 6, column 1). In contrast, 783 (92%) of the male-predominant genes showed no significant response to the loss of STAT5b in female liver (column 3). Many of the female-predominant genes showed an opposite, albeit less dramatic, response to the loss of STAT5b. Almost 62% of the female-predominant genes (464/753) responded to the loss of STAT5b in males (Fig. 2B), and in all but three of those cases, expression was increased in the absence of STAT5b (Table 6, column 1). Overall, in STAT5b-deficient male liver, 83% of the genes that were down-regulated were male-predominant genes in WT mice, whereas 68% of the up-regulated genes were female predominant (Table 7, columns 2 and 4). No clear pattern of sex-dependent regulation was seen in STAT5b-deficient female liver, where the number of genes affected by the loss of

Table 1. Sex-Specific Liver Genes that Are Up- or Down-Regulated in STAT5b-Deficient Males But Not Females and Are Not Sex Specific in the STAT5b KO Strain

A, Shown are the top 25 male-specific genes representing group 1A, the largest cluster, comprised of 560 male-specific genes. B, The top 25 female-specific genes representing group 1B, the largest cluster of female-specific genes (357 genes). MMT numbers are identification numbers assigned to probes that could not be linked to a specific GenBank ID. MMT entries are assigned the the unknown category. "Sim" in the *last column* indicates genes showing sequence similarity to the MMT probe sequence. Expression ratios shown are average values based on three independent microarrays, each carried out in duplicate (dye swap). Values shown in *bold* are significant by *P* value.

RefSeq/ GenBank ID	Common Name(s)	Description	Category	Expression Ratio (average) M-WT: F-WT	M-WT: M-KO	F-KO: F-WT	F-KO: M-KO
Group 1A. Male predominant and decreased in STAT5b-deficient males (560 genes)							
NM_021509	Moxd1,MNCb-5203	monooxygenase, DBH-like 1	metabolism	**73.11**	**79.01**	1.00	1.01
MMT00007367	MMT00007367	Sim:ril4930439M07 unclassifiable	unknown	**16.13**	**13.39**	0.91	0.88
NM_144823	Acsl6,LACS,Facl6,Lacsl	fatty acid Coenzyme A ligase, long chain 6	metabolism	**16.04**	**10.77**	0.57	0.79
MMT00053066	MMT00053066	Sim:ril4930439M07 unclassifiable	unknown	**8.76**	**7.17**	0.84	0.94
MMT00086154	MMT00086154	Sim: histocompatibility 2, class II antigen E beta	unknown	**8.63**	**8.71**	0.94	0.96
NM_133894	9430041C03Rik	RIKEN cDNA 9430041C03 gene	metabolism	**8.61**	**8.89**	0.71	0.56
MMT00019590	MMT00019590	Sim:NM_008294, hydroxysteroid dehydrogenase-4, delta<5>-3β	unknown	**6.50**	**6.69**	0.87	0.91
NM_145592	Dkk4,Dkk-4,MGC25705	dickkopf homolog 4 (Xenopus laevis)	secreted protein	**6.43**	**5.27**	1.01	1.06
NM_023617	AOH1,Aoh2	RIKEN cDNA 1200011D03 gene	metabolism	**5.25**	**5.21**	0.72	0.68
NM_007703	Elovl3,CIN-2,Cig30	elongation of very long chain fatty acids (FEN1/Elo2, SUR4)-like 3	metabolism	**5.17**	**3.90**	1.13	0.77
MMT00052306	MMT00052306	Sim:NM_010357, Mus musculus glutathione S-transferase Gsta4	unknown	**5.11**	**4.88**	1.03	1.00
NM_009235	Sox15	SRY-box containing gene 15	transcription	**4.75**	**3.55**	0.85	0.69
AK089405	2610307O08Rik	RIKEN cDNA 2610307O08 gene	unknown	**4.75**	**3.67**	0.78	0.70
NM_011304	Ruvbl2,p47,mp47	RuvB-like protein 2	apoptosis/replication	**4.69**	**3.40**	0.89	0.77
NM_198674	LOC382109	similar to hypothetical protein E330023O18	unknown	**4.40**	**3.35**	0.96	0.80
MMT00051994	MMT00051994	Sim:NM_018660, papillomavirus regulatory factor PRF-1	unknown	**4.25**	**3.56**	0.84	0.89
MMT00002293	MMT00002293	Sim:NM_002276, keratin 19	unknown	**4.13**	**3.28**	0.82	0.66
NM_021407	Trem3	triggering receptor expressed on myeloid cells 3	receptor	**4.08**	**4.05**	0.96	0.99
AF435926	Mass1,VLGR1,Frings	Mus musculus very large G protein-coupled receptor 1 (Vlgr1)	receptor	**4.06**	**3.59**	1.23	1.09
MMT00004338	MMT00004338	Sim:ril6720436F11 ribosomal protein L5	unknown	**3.98**	**3.44**	0.87	0.80
AK004863	Adh6-ps1,Adh5ps	alcohol dehydrogenase 6 (class V), pseudogene 1	unknown	**3.94**	**3.65**	0.98	0.94
AK028032	MMT00025780	product:hypothetical Kazal-type serine protease inhibitor domain	unknown	**3.91**	**3.72**	1.00	1.06
NM_010628	Kif9	kinesin family member 9	trafficking	**3.87**	**3.25**	0.92	0.77
NM_013541	Gstp1,GstpiB	glutathione S-transferase, pi 2	metabolism	**3.79**	**3.56**	0.85	0.72
NM_029444	4921504I02Rik	RIKEN cDNA 4930447C04 gene	unknown	**3.74**	**2.83**	0.82	0.69
Group 1B. Female predominant and increased in STAT5b-deficient males (357 genes)							
NM_010000	Cyp2b9	cytochrome P450, family 2, subfamily b, polypeptide 9	metabolism	**0.02**	**0.02**	0.41	0.41
AK014427	3830408G10Rik	RIKEN cDNA 3830408G10 gene	unknown	**0.05**	**0.10**	0.98	1.92
NM_010011	Cyp4a10, D4Rp1	cytochrome P450, family 4, subfamily a, polypeptide 10	metabolism	**0.10**	**0.09**	1.46	1.25
NM_007809	Cyp17a1,p450c17	cytochrome P450, family 17, subfamily a, polypeptide 1	metabolism	**0.10**	**0.11**	1.61	1.56
NM_026947	1810022C23Rik	RIKEN cDNA 1810022C23 gene	metabolism	**0.11**	**0.13**	1.85	2.65
NM_008655	Gadd45b,Myd118	growth arrest and DNA-damage-inducible 45 beta	replication	**0.12**	**0.10**	1.18	1.03
NM_018887	Cyp39a1	cytochrome P450, family 39, subfamily a, polypeptide 1	metabolism	**0.12**	**0.14**	0.79	0.85
NM_023135	Sult1e1,Ste	sulfotransferase, estrogen preferring	metabolism	**0.13**	**0.07**	1.69	0.69
NM_010924	Nnmt	nicotinamide N-methyltransferase	metabolism	**0.14**	**0.13**	0.76	0.66
NM_011076	Abcb1a,MDR3,Pgy3,Abcb4,Mdr1a	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	transporter	**0.18**	**0.11**	1.75	1.10
NM_009789	Calb3,CABP1,Cabp9k,CaBP-D9K	calbindin 3, (vitamin D-dependent calcium binding protein)	transporter	**0.18**	**0.12**	1.20	0.64
NM_010003	Cyp2c39	cytochrome P450, family 2, subfamily c, polypeptide 39	metabolism	**0.18**	**0.19**	1.26	1.23
NM_030687	Slco1a4,Oatp2,Slc21a5	solute carrier organic anion transporter family, member 1a4	transporter	**0.21**	**0.20**	1.14	1.09
NM_133994	Gstt3	glutathione S-transferase, theta 3	metabolism	**0.22**	**0.21**	1.30	1.26
NM_181409	BC051083,CRA,MGC59008	cDNA sequence BC051083	unknown	**0.22**	**0.37**	0.85	1.44
AB086957	Rfx4, MMT00021946	product:regulatory factor X, 4 (influences HLA class II expression)	transcription	**0.22**	**0.19**	1.12	0.94
NM_029494	Rsb30,5033421K01Rik	RIKEN cDNA 5033421K01 gene	trafficking	**0.25**	**0.34**	0.80	1.09
NM_009014	Rad51l1,R51H2,mREC2,Rad51b	RAD51-like 1 (S. cerevisiae)	apoptosis/replication	**0.26**	**0.19**	1.16	0.85
NM_007631	Ccnd1,Cyl-1,PRAD1,bcl-1	cyclin D1	replication	**0.28**	**0.32**	1.03	1.20
MMT00065766	MMT00065766	Sim:M21855, Mouse testosterone 16-alpha-hydroxylase	unknown	**0.29**	**0.36**	0.58	0.59
NM_008059	G0s2	G0/G1 switch gene 2	replication	**0.30**	**0.25**	1.58	1.34
NM_029537	6530411B15Rik	RIKEN cDNA 6530411B15 gene	unknown	**0.30**	**0.31**	1.14	1.18
NM_178933	Ostb	organic solute transporter beta	transporter	**0.31**	**0.14**	1.83	0.82
D17583	Pcsk5,PC6,SPC6	proprotein convertase subtilisin/kexin type 5	protein synthesis/metab	**0.32**	**0.40**	0.88	1.09
NM_145711	Tox,1700007F02Rik	thymocyte selection-associated HMG box gene	transcription	**0.32**	**0.30**	1.06	0.98

Table 2. Sex-Specific Liver Genes that Are Up- or Down-Regulated in STAT5b-Deficient Males and Females and Maintain Partial Sex Specificity in the STAT5b-KO Strain

A, The top 25 male-specific genes representing group 2A, the fourth largest cluster of male-specific genes (n = 44). B, The top 25 female-specific genes representing group 2B, the fourth largest cluster of female-specific genes (n = 39). MMT numbers and expression ratios are as described Table 1. All values shown are significant by *P* value.

RefSeq/ GenBank ID	Common Name(s)	Description	Category	Expression Ratio (average) M-WT: F-WT	M-WT: M-KO	F-KO: F-WT	F-KO: M-KO
Group 2A. Male predominant in WT and KO mice and decreased in STAT5b-deficient males and females (44 genes)							
NM_008295	Hsd3b5	hydroxysteroid dehydrogenase-5, delta<5>-3-beta	metabolism	22.30	27.59	0.07	0.26
NM_144796	E430021N18Rik,MGC30868	RIKEN cDNA E430021N18 gene	unknown	13.45	8.78	0.42	0.26
NM_145510	E130318E12Rik	RIKEN cDNA E130318E12 gene	signal transduction	11.57	6.75	0.46	0.26
AK034154	C030032C09Rik	RIKEN cDNA C030032C09 gene	signal transduction	10.94	5.43	0.54	0.39
NM_146223	Lman1l,ERGL,CPXIII,ERGIC-53L	lectin, mannose-binding, 1 like	cell adhesion	8.91	5.27	0.40	0.36
NM_054069	Psbpc1	prostatic steroid binding protein C1	secreted protein	8.35	4.95	0.49	0.38
MMT00069316	MMT00069316	Sim:M16358, Mouse major urinary protein IV (MUP IV)	unknown	7.46	4.38	0.40	0.23
NM_013797	Slco1a1,Oatp1,Slc21a1	solute carrier organic anion transporter family, member 1a1	transporter	5.92	13.32	0.16	0.39
NM_031188	Mup1,Up-1,Ltn-1,Mup-1,Mup-a	major urinary protein 1	secreted protein	5.87	2.69	0.33	0.29
NM_175475	Cyp26b1,P450RAI-2	cytochrome P450, family 26, subfamily b, polypeptide 1	metabolism	5.84	4.04	0.48	0.35
NM_177826	A930021O22	hypothetical protein A930021O22	unknown	5.72	3.74	0.65	0.49
NM_172819	AI317237,KIAA1463,4932422C22	expressed sequence AI317237	unknown	5.48	2.91	0.55	0.35
MMT00080694	MMT00080694	Sim:M16358, Mouse major urinary protein IV (MUP IV)	unknown	5.48	3.36	0.36	0.22
NM_010006	Cyp2d9	cytochrome P450, family 2, subfamily d, polypeptide 9	metabolism	5.25	3.27	0.45	0.18
NM_029550	Keg1,GS4059,0610008P16Rik	kidney expressed gene 1	metabolism	5.18	4.90	0.67	0.64
NM_008648	Mup4	major urinary protein 4	secreted protein	5.06	5.34	0.37	0.34
AI464178	6330576A10Rik	Adult male medulla oblongata cDNA product:unknown	unknown	4.60	3.67	0.66	0.61
NM_133882	C8b,4930439B20Rik	complement component 8, beta subunit	secreted protein	4.55	3.27	0.39	0.28
NM_024437	Nudt7,1300007B24Rik	nudix (nucleoside diphosphate linked moiety X)-type motif 7	metabolism	4.13	3.53	0.63	0.52
NM_013485	C9	complement component 9	secreted protein	3.93	2.45	0.63	0.39
NM_011222	Pvt1	plasmacytoma variant translocation 1	unknown	3.80	3.12	0.70	0.60
AK032187	A230052E19Rik,6430408L10	RIKEN cDNA A230052E19 gene	signal transduction	3.80	2.63	0.63	0.25
NM_010845	Mup3,MUP15,MUP III	major urinary protein 3	secreted protein	3.66	3.03	0.23	0.16
NM_007912	Egfr,Wa5,wa2,Erbb,wa-2	epidermal growth factor receptor	receptor	3.50	8.84	0.19	0.48
NM_027725	4933429D11Rik,4930563E19Rik	RIKEN cDNA 4933429D11 gene	unknown	3.38	2.22	0.67	0.44
Group 2B. Female predominant in WT and KO mice and increased in STAT5b-deficient males and females (39 genes)							
NM_019545	Hao3	hydroxyacid oxidase (glycolate oxidase) 3	metabolism	0.01	0.01	1.67	2.04
MMT00047024	MMT00047024	Sim:L27121, Mus musculus (10-1) OH-steroid sulfotrans mSTa2	unknown	0.02	0.02	2.64	5.19
AI526781	Sth2	sulfotransferase, hydroxysteroid preferring 2	metabolism	0.02	0.03	2.32	2.50
NM_007820	Cyp3a16	P450 monooxygenase Cyp3a16	metabolism	0.07	0.18	2.39	7.78
MMT00065770	MMT00065770	Sim:NM_133657, cytochrome P450, 2a12	unknown	0.08	0.05	3.52	2.10
NM_178765	5730410E15Rik	RIKEN cDNA 5730410E15 gene	unknown	0.16	0.17	2.43	2.71
BB153684	A130015N09Rik	RIKEN cDNA A130015N09 gene	transporter	0.16	0.17	2.35	2.36
NM_028545	1700055N04Rik	RIKEN cDNA 1700055N04 gene	unknown	0.18	0.19	1.64	1.80
NM_010001	Cyp2c37	cytochrome P450, family 2, subfamily c, polypeptide 37	metabolism	0.21	0.27	1.48	1.93
NM_008935	Prom1,Prom,AC133,CD133	prominin 1	cell adhesion	0.25	0.32	2.45	3.24
MMT00046397	MMT00046397	Sim:L02335, Mouse sulfotransferase (mSTa1)	unknown	0.25	0.21	3.03	2.43
NM_018881	Fmo2,MGC28212	flavin containing monooxygenase 2	metabolism	0.26	0.40	1.36	2.22
NM_011704	Vnn1,V-1	vanin 1	metabolism	0.27	0.24	2.70	2.28
NM_016869	Corin,Lrp4	corin protein	protein synthesis/metab	0.31	0.36	1.59	1.69
AK002772	1500036F01Rik	RIKEN cDNA 1500036F01 gene	unknown	0.32	0.65	1.71	3.52
NM_013743	Pdk4	pyruvate dehydrogenase kinase, isoenzyme 4	signal transduction	0.33	0.31	2.16	1.96
NM_134247	Pte2b,Pte1b,PTE-Ib	peroxisomal acyl-CoA thioesterase 2B	metabolism	0.33	0.38	1.68	1.95
NM_012006	Cte1,ACH2,CTE-1,D12Ucla1	cytosolic acyl-CoA thioesterase 1	metabolism	0.35	0.42	1.53	1.88
MMT00004272	MMT00004272	Sim: malate dehydrogenase, mitochondrial	unknown	0.36	0.26	2.49	1.86
NM_010741	Ly6c,Ly-6C	lymphocyte antigen 6 complex, locus C	cell adhesion	0.38	0.55	1.73	2.47
NM_053090	Drctnnb1a	down-regulated by Ctnnb1, a	unknown	0.43	0.42	1.63	1.62
NM_010939	Nrp2,NP2,Npn-2	neuropilin 2	cell adhesion	0.45	0.55	1.30	1.63
NM_013872	Pmm1	phosphomannomutase 1	metabolism	0.48	0.40	1.67	1.43
NM_007437	Aldh3a2,Ahd3,Aldh4,Ahd-3r	aldehyde dehydrogenase family 3, subfamily A2	metabolism	0.51	0.43	1.56	1.34
NM_026183	1300013J15Rik	RIKEN cDNA 1300013J15 gene	unknown	0.53	0.51	1.30	1.25

Table 3. Sex-Specific Genes that Are Up- or Down-Regulated in STAT5b-Deficient Males But Not Females and Maintain Partial Sex Specificity in the STAT5b-KO Strain

A, The top 25 male-specific genes representing group 3A, the second largest cluster of male-specific genes (n = 139). B, The top 25 female-specific genes representing group 3B, the fifth largest cluster of female-specific genes (n = 36). MMT numbers and expression ratios are as described in Table 1. Values in *bold* are significant by *P* value.

RefSeq/ GenBank ID	Common Name(s)	Description	Category	Expression Ratio (average) M-WT: F-WT	M-WT: M-KO	F-KO: F-WT	F-KO: M-KO
Group 3A. Male predominant in WT and KO mice and decreased in STAT5b-deficient males (139 genes)							
NM_023455	Cml4,0610037O16Rik	camello-like 4	metabolism	**18.74**	**6.88**	1.29	**0.31**
NM_028903	4933425F03Rik,4932433F15Rik	RIKEN cDNA 4933425F03 gene	unknown	**15.15**	**5.40**	0.56	**0.19**
NM_012050	Omd,SLRR2C	osteomodulin	cell adhesion	**14.08**	**3.67**	0.71	**0.18**
NM_172306	Cyp4a12	cytochrome P450, family 4, subfamily a, polypeptide 12	metabolism	**12.17**	**6.09**	1.04	**0.44**
X84013	Lama3,[a]3B,Lama3B	Similar to alpha 3A chain of laminin-5 (LOC381158), mRNA	cell adhesion	**10.54**	**4.65**	0.94	**0.62**
NM_138313	Bmf	expressed sequence AW260063	replication	**9.92**	**5.63**	0.48	**0.27**
MMT00042937	MMT00042937	Sim:weakly similar to mouse SUMO-1/SMT3-specific isopeptidase 2	unknown	**9.68**	**6.06**	0.54	**0.33**
NM_028292	Pme1, 2700017M01Rik	RIKEN cDNA 2700017M01 gene	signal transduction	**9.59**	**5.86**	0.60	**0.34**
NM_015760	Nox4	NADPH oxidase 4	metabolism	**9.41**	**4.11**	0.71	**0.42**
AK010406	2410004P03Rik	RIKEN cDNA 2410004P03 gene	unknown	**9.27**	**5.21**	0.57	**0.36**
NM_175381	2700081O15Rik	RIKEN cDNA 2700081O15 gene	unknown	**8.28**	**5.56**	0.67	**0.50**
MMT00008902	MMT00008902	Sim:AF466769, Mus musculus monoclonal antibody BBK-2 heavy chain	unknown	**7.75**	**4.87**	0.69	**0.45**
NM_016875	Ybx2,Msy2	Y box protein 2	protein synthesis/metab	**6.99**	**4.75**	0.70	**0.48**
AK019538	4921511M17Rik	RIKEN cDNA 4921511M17 gene	unknown	**6.58**	**3.98**	0.62	**0.46**
NM_007837	Ddit3,chop,CHOP-10,gadd153	DNA-damage inducible transcript 3	transcription	**6.56**	**4.30**	0.72	**0.52**
MMT00010153	MMT00010153	Sim:similar to squamous cell carcinoma antigen 2 [Mus musculus]	unknown	**6.40**	**4.50**	0.72	**0.51**
NM_153073	Gab3,5930433H21Rik	growth factor receptor bound protein 2-associated protein 3	signal transduction	**6.37**	**4.40**	0.71	**0.55**
MMT00034132	MMT00034132	Sim:AF106620, mitochondrial inner membr translocase Tim17a	unknown	**6.32**	**3.72**	0.73	**0.50**
NM_172945	B930093C12Rik	RIKEN cDNA B930093C12 gene	unknown	**6.14**	**4.33**	0.71	**0.51**
NM_177787	9830102E05	hypothetical protein 9830102E05	unknown	**6.03**	**4.21**	0.96	**0.70**
NM_029160	Spag16, 4921511D23Rik	Sperm associated antigen 16 (Spag16), transcript variant 1	cytoskeleton	**5.88**	**3.69**	0.77	**0.54**
AK077141	4930438O03Rik	product:hypothetical MORN motif containing protein	unknown	**5.65**	**3.87**	0.72	**0.56**
NM_027017	3300002I08Rik,2900016H11Rik	KRAB-Zfp119-like, RIKEN cDNA 3300002I08 gene	transcription	**5.48**	**4.05**	0.71	**0.53**
NM_009653	Alas2,ALAS,Alas-2	aminolevulinic acid synthase 2, erythroid	metabolism	**5.42**	**3.53**	1.11	**0.68**
NM_054042	Cd164l1,Tem1,2610111G01Rik	CD164 sialomucin-like 1	cell adhesion	**5.02**	**3.96**	0.86	**0.70**
Group 3B. Female predominant in WT and KO mice and increased in STAT5b-deficient males (36 genes)							
NM_020565	Sult3a1,Sultx2,Sult-x2	sulfotransferase related gene X2	metabolism	**0.01**	**0.03**	1.18	**10.32**
AI509050	A1bg,C44	Similar to α-1-B glycoprotein; liver regeneration-related protein	secreted protein	**0.02**	**0.24**	1.27	**25.71**
NM_146232	BC014805,MGC25980	cDNA sequence BC014805	transporter	**0.02**	**0.02**	0.99	**2.03**
MMT00047061	MMT00047061	Sim:L27121, Mus musculus (10-1) OH-steroid sulfotrans mSTa2	unknown	**0.03**	**0.05**	2.21	**2.48**
NM_011994	Abcd2,ALDR,ABC39,ALDL1	ATP-binding cassette, sub-family D (ALD), member 2	transporter	**0.07**	**0.08**	1.31	**1.48**
NM_145368	C730036D15Rik	RIKEN cDNA C730036D15 gene	metabolism	**0.07**	**0.13**	0.82	**2.00**
NM_134246	Pte2a,PTE-Ia	peroxisomal acyl-CoA thioesterase 2A	metabolism	**0.10**	**0.13**	1.72	**2.49**
NM_007804	Cutl2,Cux2,Cux-2	cut-like 2 (Drosophila)	transcription	**0.12**	**0.31**	0.99	**2.69**
NM_029682	AMSH-FP, ALMalpha	Associated molecule with SH3 domain of STAM (AMSH)-like protein	signal transduction	**0.24**	**0.31**	1.12	**1.45**
NM_145076	TIF1	Tripartite motif protein 24 (TIF1, Trim24).	transcription	**0.27**	**0.42**	0.95	**1.45**
NM_024198	Gpx7,GPX6,3110050F08Rik	RIKEN cDNA 3110050F08 gene	metabolism	**0.29**	**0.64**	0.79	**1.78**
NM_009876	Cdkn1c,CDKI,Kip2,p57Kip2	cyclin-dependent kinase inhibitor 1C (P57)	replication	**0.31**	**0.38**	1.32	**1.63**
NM_008932	Prlr	prolactin receptor	receptor	**0.39**	**0.54**	1.25	**1.78**
NM_008706	Nqo1,Ox1,QR1,Dia4,NMO1,Nmor1	NAD(P)H dehydrogenase, quinone 1	metabolism	**0.40**	**0.41**	1.20	**1.26**
NM_007620	Cbr1,CR,Cbr	carbonyl reductase 1	metabolism	**0.40**	**0.54**	1.08	**1.48**
NM_011125	Pltp	phospholipid transfer protein	metabolism	**0.40**	**0.58**	1.50	**2.14**
NM_009801	Car2,CAII,Car-2,Ltw-5,Lvtw-5	carbonic anhydrase 2	metabolism	**0.42**	**0.75**	1.05	**1.96**
NM_011851	Nt5e,NT,Nt5,eNT,CD73	5′ nucleotidase, ecto	metabolism	**0.46**	**0.51**	1.16	**1.30**
NM_018884	Pdzrn3,LNX3,Semcap3	RIKEN cDNA 1110020C07 gene	signal transduction	**0.47**	**0.58**	1.03	**1.32**
NM_130862	Baiap2,IRSp53	brain-specific angiogenesis inhibitor 1-associated protein 2	cytoskeleton	**0.47**	**0.71**	1.01	**1.51**
NM_032541	Hamp,Hepc,HEPC1	hepcidin antimicrobial peptide	secreted protein	**0.49**	**0.65**	1.26	**1.64**
AK041121	2610301F02Rik	RIKEN cDNA 2610301F02 gene	unknown	**0.49**	**0.79**	0.98	**1.54**
BC023460	2310031A18Rik	RIKEN cDNA 2310031A18 gene	unknown	**0.52**	**0.66**	1.13	**1.46**
NM_029653	Dapk1,D13Ucla1,DAP-Kinase	death associated protein kinase 1	replication	**0.53**	**0.64**	**1.18**	**1.45**
NM_027222	2010001M09Rik	RIKEN cDNA 2010001M09 gene	unknown	**0.53**	**0.64**	1.11	**1.32**

Table 4. Distribution of 2135 of the 2231 Differentially Expressed Genes within 22 Gene Groups

Group	Total Flagging Sum (TFS)	No. of Genes	Sex Specificity (WT)	Sex Specificity (KO)	Direction of Regulation by STAT5b in Males	Direction of Regulation by STAT5b in Females
1A	3.2200	560	M	–	Up	–
1B	3.1100	357	F	–	Down	–
2A	15.2211	44	M	M	Up	Up
2B	15.1122	39	F	F	Down	Down
3A	11.2201	139	M	M	Up	–
3B	11.1102	36	F	F	Down	–
4A	1.2000	58	M	–	–	–
4B	1.1000	229	F	–	–	–
5A	9.2001	20	M	M	–	–
5B	9.1002	43	F	F	–	–
6A	7.2210	15	M	–	Up	Up
6B	7.1120	15	F	–	Down	Down
7A	8.0001	39	–	M	–	–
7B	8.0002	127	–	F	–	–
8A	2.0200	86	–	–	Up	–
8B	2.0100	133	–	–	Down	–
9A	4.0010	30	–	–	–	Up
9B	4.0020	17	–	–	–	Down
10A	6.0210	36	–	–	Up	Up
10B	6.0120	21	–	–	Down	Down
11	10.0101	51	–	M	Down	–
12	12.0022	40	–	F	–	Down

A total of 2135 genes are described. Genes are grouped based on the flagging system described in *Materials and Methods*. Group numbers correspond to the gene group designations detailed in Tables 1–3 and supplemental Table 1. The Total Flagging Sum (TFS) indicates the direction of response for each ratio, which is detailed in the *four rightmost columns* as indicated by male (M), female (F), and up- (Up) or down-regulation (Down) in wild-type and STAT5b-knockout males or females, or no response (–), as indicated. Twenty-five gene groups, each containing fewer than 15 genes, and together comprising only 96 (4%) of the 2231 genes of interest, were not assigned group numbers and are not shown.

STAT5b was far fewer than in males (Table 8). The sex-dependent role of STAT5b was also apparent when we considered the 1715 genes the expression of which was altered by the loss of STAT5b in either males or females (Table 9). A full 1391 (81%) of these genes showed regulation in male liver only, whereas only 119 genes (7%) showed regulation in female liver only, and 205 (12%) showed regulation in both sexes (Table 9, *last line*). Overall, *Stat5b* disruption resulted in a substantial loss of sexual dimorphism, with only 24% (206) of the male-predominant genes and 17% (125) of the female-predominant genes retaining their normal sexual dimorphism in the absence of STAT5b (Table 10).

Quantitative Relationships Revealed by Array-Array Comparisons

Genes displaying significant differential expression in any two of the four microarray experiments were examined for quantitative correlations between experiments. Average $\log_2$ expression ratios for each gene were plotted on a log-log scale, and linear regression was used to determine the degree of correlation of the expression ratios between microarrays. A correlation coefficient and slope close to 1 indicates very high similarity between two gene expression profiles, whereas a low correlation between two profiles means no commonality likely exists. The most highly correlated relationship among the six intermicroarray comparisons was obtained for the M-WT:F-WT *vs.* M-WT:M-KO comparisons (Fig. 3A). A total of 1232 genes met the threshold criteria for both sets of microarrays. Male-predominant genes showed a loss of expression in the absence of STAT5b in male liver, and female-predominant genes showed a gain of expression in male liver upon the loss of STAT5b, with the exception of only four genes (data points in quadrants II and IV; Fig. 3A). The extent to which these 1232 genes responded to the loss of STAT5b in males was strongly correlated to the magnitude of their sex specificity (slope = 0.9; y-intercept = 0.03; r = 0.966). This provides a strong indication that STAT5b is a primary determinant of sex-specific expression in male mouse liver.

A second relationship with a high correlation was seen for the 346 genes that showed sex specificity in both WT and STAT5b-KO mouse strains (Fig. 3B). The magnitude of the sex specificity of these genes in STAT5b-deficient mouse liver was lower than that in WT mice, as indicated by the slope of the best-fit line being less than 1 (slope = 0.46; y-intercept = 0.005; r = 0.811). Thus, not only do a large number of genes lose sex specificity in the absence of STAT5b, but even in the case of the 346 genes that retain sex

Table 5. *Mup* (A) and *Cyp* (B) Family Members within the 2231 Differentially Expressed Genes

RefSeq/ GenBank ID	Common Names	Description	Group	Expression Ratio (Average)			
				M-WT: F-WT	M-WT: M-KO	F-KO: F-WT	F-KO: M-KO
A. *Mup* family members within the 2231 differentially expressed genes							
NM_031188	Mup1, Up-1, Ltn-1, Mup-a	Major urinary protein 1	2A	**5.87**	**2.69**	**0.33**	**0.29**
NM_010845	Mup3, MUP15, MUP III	Major urinary protein 3	2A	**3.66**	**3.03**	**0.23**	**0.16**
NM_008648	Mup4	Major urinary protein 4	2A	**5.06**	**5.34**	**0.37**	**0.34**
MMT00069295	MMT00069295	Sim:M16355, Mouse major urinary protein I (MUP I)	2A	**2.88**	**2.67**	**0.26**	**0.21**
MMT00080694	MMT00080694	Sim:M16358, Mouse major urinary protein IV (MUP IV)	2A	**5.48**	**3.36**	**0.36**	**0.22**
MMT00069316	MMT00069316	Sim:M16358, Mouse major urinary protein IV (MUP IV)	2A	**7.46**	**4.38**	**0.4**	**0.23**
B. *Cytochrome P450* gene superfamily members within the 2231 differentially expressed genes							
NM_007814	Cyp2b19	Cytochrome P450, family 2, subfamily b, polypeptide 19	1A	**2.33**	**2.02**	1.05	0.97
MMT00059415	MMT00059415	Sim:AB084894, Rattus norvegicus CYP3A mRNA	1A	**1.98**	**1.69**	0.96	0.88
NM_010000	Cyp2b9	Cytochrome P450, family 2, subfamily b, polypeptide 9	1B	**0.02**	**0.02**	0.41	0.41
NM_009998	Cyp2b10	Cytochrome P450, family 2, subfamily b, polypeptide 20	1B	**0.33**	**0.25**	1.30	1.00
NM_010003	Cyp2c39	Cytochrome P450, family 2, subfamily c, polypeptide 39	1B	**0.18**	**0.19**	1.26	1.23
NM_134144	Cyp2c50	Cytochrome P450, family 2, subfamily c, polypeptide 50	1B	**0.66**	**0.56**	1.00	0.80
NM_206537	Cyp2c54	Cytochrome P450, family 2, subfamily c, polypeptide54	1B	**0.54**	**0.50**	0.74	0.67
NM_017396	Cyp3a41	Cytochrome P450, family 3, subfamily a, polypeptide 41	1B	**0.33**	**0.37**	1.10	1.24
NM_018887	Cyp39a1	Cytochrome P450, family 39, subfamily a, polypeptide 1	1B	**0.12**	**0.14**	0.79	0.85
NM_010011	Cyp4a10	Cytochrome P450, family 4, subfamily a, polypeptide 10	1B	**0.10**	**0.09**	1.46	1.25
NM_024444	Cyp4f18	Cytochrome P450, family 4, subfamily f, polypeptide 18	1B	**0.63**	**0.63**	1.02	1.02
NM_007809	Cyp17a1	Cytochrome P450, family 17, subfamily a, polypeptide 1	1B	**0.10**	**0.11**	1.61	1.56
MMT00065736	MMT00065736	Sim:NM_007813, cytochrome P450 Cyp2b13	1B	**0.36**	**0.46**	0.64	0.71
NM_010006	Cyp2d9	Cytochrome P450, family 2, subfamily d, polypeptide 9	2A	**5.25**	**3.27**	**0.45**	**0.18**
NM_175475	Cyp26b1	Cytochrome P450, family 26, subfamily b, polypeptide 1	2A	**5.84**	**4.04**	**0.48**	**0.35**
NM_010001	Cyp2c37	Cytochrome P450, family 2. subfamily c, polypeptide 37	2B	**0.21**	**0.27**	**1.48**	**1.93**
NM_007820	Cyp3a16	Cytochrome P450, family 3, subfamily a, polypeptide 16	2B	**0.07**	**0.18**	**2.39**	**7.78**
MMT00065770	MMT00065770	Sim:NM_133657, cytochrome P450, 2a12	2B	**0.08**	**0.05**	**3.52**	**2.10**
NM_172306	Cyp4a12	Cytochrome P450, family 4, subfamily a, polypeptide 12	3A	**12.17**	**6.09**	1.04	**0.44**
NM_001003947	Cyp4 × 1	Cytochrome P450, family 4, subfamily x, polypeptide 1	3A	**1.75**	**1.65**	0.89	**0.82**
NM_010004	Cyp2c40	Cytochrome P450, family 2, subfamily c, polypeptide 40	3B	**0.64**	**3.11**	0.84	**4.02**
NM_007815	Cyp2c29	Cytochrome P450, family 2, subfamily c, polypeptide 29	5A	**1.58**	0.78	1.26	**0.62**
NM_009995	Cyp21a1	Cytochrome P450, family 21, subfamily a, polypeptide 1	5B	**0.61**	0.85	1.20	**1.74**
NM_007825	Cyp7b1	Cytochrome P450, family 7, subfamily b, polypeptide 1	6A	**8.93**	**13.41**	**0.39**	0.66
MMT00045463	MMT00045463	Sim:AF204959, Mus musculus cytochrome P450 3A25	7A	1.48	1.09	0.78	**0.58**
NM_010002	Cyp2c38	Cytochrome P450, family 2, subfamily c, polypeptide 38	8B	0.96	**0.53**	1.25	0.70
NM_007818	Cyp3a11	Cytochrome P450, family 3, subfamily a, polypeptide 11	8B	0.51	**0.47**	1.05	0.97
NM_028089	Cyp2c55	Cytochrome P450, family 2, subfamily c, polypeptide 55	11	1.34	**0.33**	1.53	**0.38**
MMT00021466	MMT00021466	Sim:NM_175766, cytochrome P450 Cyp2J3	11	1.11	**0.68**	0.98	**0.60**
NM_007813	Cyp2b13	Cytochrome P450, family 2, subfamily b, polypeptide 13	–	**0.02**	**0.01**	0.39	**0.35**
NM_007822	Cyp4a14	Cytochrome P450, family 4, subfamily a, polypeptide 14	–	**0.45**	**0.36**	0.93	**0.62**
NM_013809	Cyp2g1	Cytochrome P450, family 2, subfamily g, polypeptide 1	–	**0.59**	1.10	**0.47**	0.88

Genes listed met the criteria described in the *Materials and Methods* and were also annotated as a member of the *Mup* or *Cyp* gene families. All six *Mup* gene probes identified genes that were male specific in both wild-type and STAT5b-knockout mice, and were up-regulated by STAT5b in both males and females (group 2A). *Cyps* belonged to multiple gene groups, as indicated. Genes with MMT numbers have no assigned GenBank ID and are listed as unknown. Ratios shown in *bold* were found to be statistically significant ($P < 0.05$). *Cyps* belonging to gene groups with fewer than 15 members were not assigned a group number.

specificity in the STAT5b-deficient mice, the extent of sex specificity is substantially reduced.

Finally, of the 125 sex-specific genes the expression of which was altered in both STAT5b-deficient males and females, all but nine of the genes responded in the same manner in both sexes, albeit not to the same degree (slope = 0.41; y-intercept = 0.15; $r = 0.809$) (Fig. 3C). A similar conclusion can be drawn from Table 9 (columns 5 and 6) by examination of the direction of response by sex for the 205 sex-specific and non-sex-specific genes that were affected by the loss of STAT5b in both males and females. All but 16 of the 205 genes responded to the loss of STAT5b in the same manner in both sexes.

The remaining three array-array comparisons are presented in supplemental Fig. 1 published as supplemental data on The Endocrine Society's Journals Online web site at http://mend.endojournals.org. The correlation of gene expression patterns for sex-dependent genes between WT female and STAT5b-KO male liver, seen in Fig. 3A, is also evident from supplemental Fig. 1A, where a strong positive correlation was observed for the genes that responded to the loss of STAT5b in females and retained

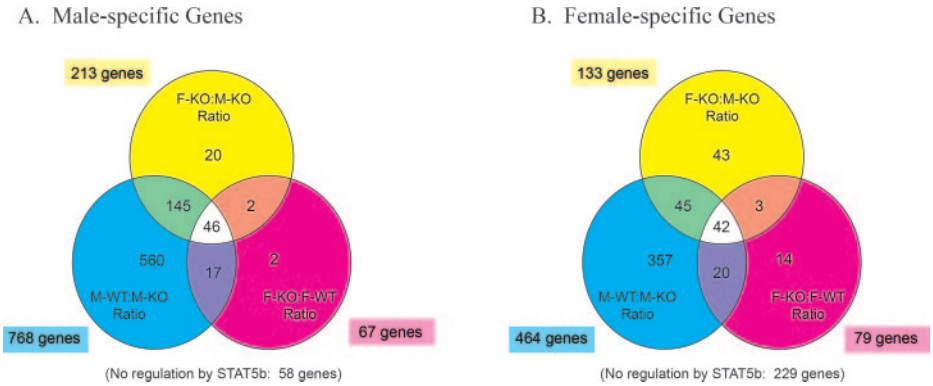

Fig. 2. Venn Diagram Representations of Liver Gene Expression Profiles for Male-Predominant and Female-Predominant Genes

A, Male-predominant genes showing sex specificity (M-WT:F-WT >1.5; $P < 0.05$) were placed into the diagram based on their differential expression (ratio >1.25 or <0.8; $P < 0.05$) among the three other microarrays. The greatest number of male-predominant genes (n = 768) showed a response to the loss of STAT5b in male liver (M-WT:M-KO ratio). B, Female-predominant genes showing sex specificity (M-WT:F-WT <0.66, $P < 0.05$) were placed into the diagram based on the same criteria as in panel A. No differential expression in any of the three other arrays was seen for 58 of the 850 male-predominant genes and for 229 of the 753 female-predominant genes; these genes are not represented in the diagram.

sex specificity in the STAT5b-KO mice (slope = 0.97; y-intercept = 0.04; $r = 0.841$). Comparison of sex-specific gene expression and response to the loss of STAT5b in females yielded a weaker correlation (n = 146; $r = 0.629$) (supplemental Fig. 1B). A similar result was obtained for the correlation between expression ratios for genes that were sex specific in the STAT5b-KO mice and also responded to the loss of STAT5b in males (n = 278; $r = 0.693$) (supplemental Fig. 1C).

Enrichment of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Terms for Sex-Dependent Genes

Using Rosetta Resolver software, we were able to annotate 1374 of the 1603 sex-dependent genes with a molecular function category containing GO or KEGG pathway information. A total of 1053 different GO categories and 117 different KEGG pathways were found within the annotations for the 1374 genes. Thirty four GO categories and 16 KEGG pathways had E values less than 1.0 (see *Materials and Methods*), indicating a highly significant enrichment for those terms within the annotations of the 1374 genes (Table 11, A and B). A large number of these GO categories are related to oxidative metabolism, including monooxygenase, peroxidase, and a variety of oxidoreductase activities. KEGG pathways related to tryptophan and fatty acid metabolism, steroid metabolism, cytokine receptors, and the JAK-STAT signaling pathway were also overrepresented within the sex-dependent gene set. Further analysis, based on curated searches of the 1603 sex-dependent genes for gene family information within the Gene database from NCBI, followed by statistical evaluation of the results, gave the results shown in Table 11C. Cytochrome P450 enzymes, sulfotransferases, serine peptidase inhibitors, and several gene families involved in foreign compound metabolism or transport were overrepresented among the sex-specific liver genes.

DISCUSSION

Oligonucleotide-based microarrays were used to investigate the sexual dimorphism of mouse liver gene expression and the role of STAT5b in maintaining this dimorphism, as revealed by the impact of global disruption of the *Stat5b* gene. This genome-wide approach complements more traditional methods, such as real-time quantitative PCR (qPCR), which has pro-

Table 6. Impact of *Stat5b* Disruption on Sex-Specific Gene Expression.

	M-WT:M-KO		F-WT:F-KO	
	Gene Count	%	Gene Count	%
Male genes				
Increase	1	<1	5	1
Decrease	767	90	62	7
No change	82	10	783	92
Total	850		850	
Female genes				
Increase	461	61	56	7
Decrease	3	<1	23	3
No change	289	38	674	90
Total	753		753	

Genes meeting the criteria described in the *Materials and Methods* were initially sorted based on sex specificity and secondarily sorted by response to STAT5b knockout in males and females. Ninety percent of male-specific genes show down-regulation in the absence of STAT5b in males, whereas 92% of the same genes show no regulation by STAT5b in females.

Table 7. Sex Specificity of Genes Responding to Loss of STAT5b in Males and in Females: Genes Responding to the Loss of STAT5b in Male Liver

Sex Specificity	Genes Down-Regulated in STAT5b KO Males		Genes Up-Regulated in STAT5b KO Males		Genes Unchanged in STAT5b KO Males		Total
	Gene Count	%	Gene Count	%	Gene Count	%	
Male	767	83	1	<1	82	13	850
Female	3	<1	461	68	289	46	753
No sex specificity	150	16	214	32	264	42	628
Total	920		676		635		2231

Genes meeting the criteria described in the *Materials and Methods* were initially sorted according to their response to STAT5b deficiency in males and secondarily sorted based on their sex specificity in wild-type liver. Eighty-three percent of the genes showing down-regulation and only one of the 676 genes showing up-regulation in the male STAT5b-knockout mice are male-predominant genes.

vided quantitative data on the impact of STAT5b deficiency on a limited number of genes, primarily *Cyp* genes, the expression of which is sex dependent in WT mouse liver, and the sex specificity of which is abolished in male mice when the endogenous, pulsatile plasma GH pattern is abolished by continuous infusion of exogenous GH (20). Earlier studies have shown that STAT5b is activated in male rat liver in direct response to each incoming plasma GH pulse and is partially down-regulated by a continuous, female-like pattern of GH stimulation, leading to the hypothesis that STAT5b functions as a mediator of the transcriptional effects of plasma GH pulsation on male liver gene expression (19). In the present study, we carried out four sets of microarray experiments comparing male and female gene expression, as well as the effects of *Stat5b* gene disruption on gene expression in males and females, enabling us to identify 2231 genes that displayed differential expression under one or more of the four sets of experimental conditions.

A large number of the differentially expressed genes (1603 of 2231; 72%) exhibited sex-dependent expression in WT mouse liver. These genes correspond to an estimated 4% of the entire mouse genome, and their sexual dimorphism helps to explain the sex differences observed in several physiological pathways, including steroid and xenobiotic metabolism, inflammatory response, and homeostasis. Female-predominant genes previously characterized in the mouse or rat liver model, and reaffirmed in the present study, include *Cyp2b9*, *Cyp2b13* (36, 37), *17β-hydroxysteroid dehydrogenase type 2* (38), *alcohol dehydrogenase* (39), *α-1B-glycoprotein*, *prolactin receptor* (40), and several *sulfotransferase* genes (41). Male-predominant genes include *glutathione S-transferase* (*GST*) *π* (36, 42), *Cyp4a12* (36, 43), *Cyp7b1* (36, 44), and several *serine protease inhibitor* genes (45). The magnitude of sex specificity for the 1603 genes identified here ranged up to 98-fold, with some of the highest sex-specificity ratios observed for certain *Cyps* (Table 5B) and other genes associated with steroid and foreign compound metabolism (supplemental Tables 2, 3, 6, 7, 8, 10, and 11), several of which have been previously studied in this regard. However, most of the sex-dependent genes identified in the present study are novel observations. Whereas genes involved in oxidative metabolism, in particular, steroid and foreign compound metabolism, were enriched within the list of sex-dependent genes, a number of other molecular functions and physiological pathways, including protein-tyrosine kinase activity, tryptophan and fatty acid metabolism, cytokine signaling, complement and coagulation cascades, and solute carrier proteins, were also overrepresented (Table 11). Thus, sex-dependent expression in the liver is common and extends beyond the genes involved in steroid hormone metabolism and reproductive behavior to include large numbers of receptors, signaling molecules and nuclear factors, several of which may conceivably contribute as medi-

Table 8. Sex Specificity of Genes Responding to Loss of STAT5b in Males and in Female: Genes Responding to the Loss of STAT5b in Female Liver

Sex Specificity	Genes Down-Regulated in STAT5b KO Females		Genes Up-Regulated in STAT5b KO Females		Genes Unchanged in STAT5b KO Females		Total
	Gene Count	%	Gene Count	%	Gene Count	%	
Male	62	35	5	3	783	41	850
Female	23	13	56	38	674	35	753
No Specificity	90	51	88	59	450	24	628
Total	175		149		1907		2231

Genes meeting the criteria described in the *Materials and Methods* were initially sorted according to their response to STAT5b deficiency in females and secondarily sorted based on their sex specificity in wild-type liver.

Table 9. Changes in Gene Expression in STAT5b-KO Mice Compared with Wild-Type Mice of the Same Sex

Comparison	Gene Expression Changes in STAT5b-KO Males Only		Gene Expression Changes in STAT5b-KO Females Only		Gene Expression Changes in STAT5b-KO Mice of Both Sexes		Total	%
	Gene Count	%	Gene Count	%	Gene Count	%		
WT < KO	586	34	61	4	81	5	728	42
WT > KO	805	47	58	3	108	6	971	57
M(WT<KO), F(WT>KO)					9	1	9	1
M(WT>KO), F(WT<KO)					7	<1	7	<1
Total	1391	81	119	7	205	12	1715	

A total of 1715 liver-expressed genes meeting the criteria described in *Materials and Methods* in either males or females of the STAT5b-knockout strain are listed according to the direction of expression change in knockout (KO) as compared with wild-type (WT) mouse liver and whether the change in expression occurs in males, females, or both sexes. Eighty-one percent of all genes showing a change of expression in any of the STAT5b-knockout mice as compared with wild-type showed a change in expression in males only. A total of 516 genes showed no change in expression in the STAT5b-knockout mice of either sex.

ators or modulators of the effects of GH on liver gene expression, as discussed below.

A high correlation between the effect of sex and *Stat5b* genotype on gene expression was seen in male mouse liver, where male-predominant genes were down-regulated in the absence of STAT5b whereas many female-predominant genes were up-regulated. The striking linear relationship ($r = 0.966$) between magnitude of sex specificity in the WT and response to the loss of STAT5b in males, combined with the high Pearson's correlation coefficient between sex specificity and response to the loss of STAT5b in males for all genes of interest, supports the conclusion that STAT5b is a key determinant of sex specificity in male liver. By contrast, far fewer sex-specific genes responded to the loss of STAT5b in females, and a poor correlation was observed among those genes that did yield a response. Direct comparison of the gene expression profiles of STAT5b-KO males and females revealed that a majority of the sex-dependent liver genes (1257 of 1603 genes; 78%) were no longer expressed in a sex-dependent manner in mice deficient in STAT5b (Table 10). STAT5b is thus essential for a major portion of the sexual dimorphism that characterizes mouse liver.

Table 10. Impact of STAT5b-KO on Male-Specific and Female-Specific Gene Expression

Sex Specificity in STAT5b KO Strain	Gene Count	%
Genes male-specific in WT		
F > M	7	1
M > F	206	24
Nonspecific	637	75
Total	850	
Genes female-specific in WT		
F > M	125	17
M > F	8	1
Nonspecific	620	82
Total	753	

A total of 1603 liver-expressed genes meeting the criteria for sex specificity in wild-type mice are listed according to the sex specificity of their expression in STAT5b-KO mice. A large majority of the genes do not retain sex specificity in STAT5b-knockout mice.

The present findings are in good agreement with a previous study in which qPCR was used to analyze the impact of STAT5b deficiency on the expression of 15 sex-specific liver genes, which could be grouped based on their sex dependence and response to the loss of STAT5b (20). Clustering of the 2231 sex- and/or STAT5b-dependent genes identified in the present study, based on their responses to each of four competitive hybridization experiments, enabled us to classify the majority of the regulated genes into 22 major groups (Table 4). Genes belonging to the largest group, 1A, were male predominant in their expression and required STAT5b for expression in males, but were unaffected by STAT5b deficiency in females (Table 1A). The expression profile of the group 1A genes is consistent with a regulatory pattern previously observed for three of seven male-predominant genes examined by qPCR, termed "class I" male genes (20). The second largest group of genes, 1B, was female predominant and apparently repressed by STAT5b in males but not females (Table 1B). Many *Cyps* belong to this group (Table 5B), several of which were previously shown to have a similar expression pattern by qPCR ("class II" male genes) (20). Together, these two groups make up 41% of the 2231 sex- or STAT5b-dependent genes reported here. It is not known whether STAT5b regulates *Cyps* and other sex-dependent liver genes by a direct transcriptional mechanism. Conceivably, STAT5b may regulate many of these genes indirectly, as discussed below.

Genes belonging to groups 2A and 2B, respectively, share the regulatory characteristics of groups 1A and 1B but, in addition, show dependence on STAT5b in females. The partial sex specificity maintained by group 2A and 2B genes in the STAT5b-KO strain suggests that factors other than STAT5b contribute to sex-specific expression. Of note, the male specificity of several group 2A genes (namely, *Cyp2d9* and sev-

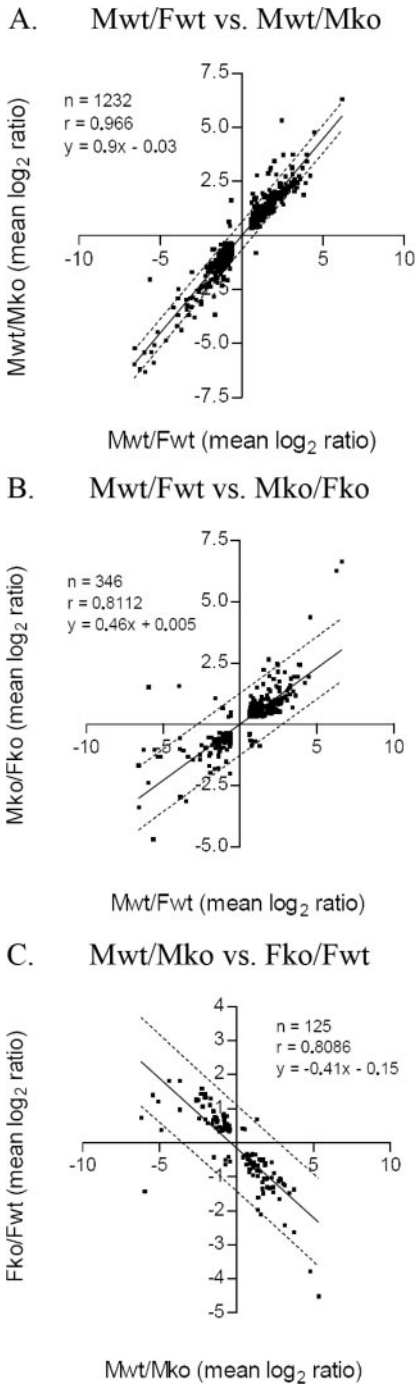

Fig. 3. Scatterplots for Quantitative Correlation of Microarray Comparisons

A, M-WT:F-WT comparison with M-WT:M-KO. Shown are $\log_2$ ratios for M-WT:F-WT (x-axis) and the corresponding M-WT:M-KO data (y-axis) plotted on a log-log scale for all genes (n =1232) where both ratios met the criteria set in *Materials and Methods* and Fig. 2. The two conditions are highly correlated ($r = 0.966$), and the best-fit line ($y = 0.9x - 0.03$), shown in *solid*, has a slope near 1 and an intercept near 0. The 95% prediction boundaries are shown as *dashed lines*. Genes found in the *upper right quadrant* (I) are male predominant and were decreased in expression in male liver in the absence of STAT5b, whereas genes found in the *lower left quadrant* (III) are female predominant and were increased in expression in STAT5b-deficient male liver. B, M-WT:F-WT comparison with M-KO:F-KO. Similar to graph A, shown are the genes (n = 346) meeting the aforementioned criteria for the M-WT:F-WT (x-axis) and M-KO:F-KO (y-axis) average $\log_2$ ratios. Genes found to be sex specific in both WT and STAT5b-KO liver retain their sex specificity in the absence of STAT5b ($r = 0.81$, $y = 0.46x + 0.005$) with only 16 outliers, represented by points in quadrants II and IV. C, M-WT:M-KO comparison with F-KO:F-WT. Shown are the responses of sex-specific genes in the WT that also meet the aforementioned criteria for the M-WT:M-KO (x-axis) and F-KO:F-WT (y-axis) ratios (n =125 genes). Genes in the *lower right quadrant* are lower in expression in both male and female livers upon loss of STAT5b. Genes in the *upper left quadrant* show higher liver expression in the absence of STAT5b in both sexes.

eral members of the *Mup* family) is, in part, enforced by KRAB zinc finger repressors of the *Rsl* family, which may act independently of STAT5b and preferentially suppress expression of these genes in female mouse liver (46–48). The finding that STAT5b regulates a subset of sex-dependent genes in female liver, in addition to male liver, confirmed by qPCR for *Cyp2d9* and the *Mup* genes (20), is consistent with the presence of nuclear STAT5b activity in females, albeit at a much lower level than in males (15). Three female-predominant *Cyp3a* genes, *Cyp3a16, Cyp3a41,* and *Cyp3a44*, were previously found by qPCR to be largely unresponsive to the loss of STAT5b (20). Up-regulation of a female-specific, Cyp3a-immunoreactive protein was previously seen in STAT5b-deficient male liver (22), as was the up-regulation of *Cyp3a16* and *Cyp3a41* RNA (Table 5B). However, neither of these genes demonstrated the same high degree of sex specificity (F $>$ M by $\geq$1000-fold) determined by qPCR (20), suggesting that their apparent up-regulation may reflect, in part, nonspecific cross-reactivity and cross-hybridization with other *Cyp3a* family members. Genes making up groups 3A and 3B were down-regulated in the absence of STAT5b in males only, yet they maintained sex specificity in the STAT5b-KO mice (Table 3). This pattern may be explained if STAT5b regulation in males is not the sole cause for the observed sex specificity. Another possible explanation is false-negative results in the F-KO:F-WT comparison, which would make it impossible to distinguish these genes from those in groups 2A and 2B.

Several other large gene groups were identified in the present study (Table 4). We identified 287 genes in two groups that are sex specific in WT mice but were not affected by the loss of STAT5b in either males or females (groups 4A and 4B). It is difficult to explain the expression profiles of these genes, insofar as they were not sex specific in the STAT5b-KO strain. The loss of STAT5b may be significant enough to alter the sex-specific expression of these genes but not enough to be detected in the direct comparisons of WT and STAT5b-KO liver samples in each sex. Two

Table 11. Enrichment for GO/KEGG Terms and Gene Families within the Sex-Specific Genes

GO Molecular Functions/KEGG Pathway/Gene Family	E Value	*P* Value	Overlap Gene Count[a]	Set Gene Count[b]
A. GO terms with E values less than 1.0 within the sex-specific genes				
Monooxygenase activity	2.45E-12	2.33E-15	31	115
Oxidoreductase activity (paired donors with incorporation or reduction of molecular oxygen)	2.50E-10	2.37E-13	27	102
Steroid hydroxylase activity	2.76E-04	2.62E-07	10	27
Protein-tyrosine kinase activity	9.87E-04	9.37E-07	38	334
Antioxidant activity	0.0018	1.71E-06	15	73
Peroxidase activity	0.0044	4.17E-06	12	51
Carboxylesterase activity	0.0056	5.30E-06	10	36
Oxidoreductase activity (acting on peroxide as acceptor)	0.0067	6.40E-06	12	53
Growth factor activity	0.0121	1.15E-05	22	162
Ion transporter activity	0.0214	2.03E-05	39	395
α-Type channel activity	0.0254	2.41E-05	39	398
Lipid binding	0.0279	2.65E-05	30	274
Serine esterase activity	0.0395	3.75E-05	8	28
Ion channel activity	0.0423	4.02E-05	36	364
Oxidoreductase activity (acting on CH-OH group of donors)	0.0429	4.08E-05	20	151
CoA hydrolase activity	0.0450	4.28E-05	6	15
Sulfotransferase activity	0.0472	4.48E-05	10	45
Channel or pore class transporter activity	0.0491	4.66E-05	39	410
Cation channel activity	0.0520	4.93E-05	28	256
Glucuronosyltransferase activity	0.0566	5.38E-05	7	22
Acyl-CoA thioesterase activity	0.1625	1.54E-04	5	12
Electron transporter activity	0.1893	1.80E-04	26	248
Sulfur-containing groups transferase activity	0.2030	1.93E-04	10	53
Oxidoreductase activity (paired flavin-related donors with incorporation of one atom of oxygen)	0.2832	2.69E-04	6	20
Protein serine/threonine kinase activity	0.2839	2.70E-04	42	492
DNA-(apurinic or apyrimidinic site) lyase activity	0.3396	3.23E-04	4	8
Testosterone 16-α-hydroxylase activity	0.3396	3.23E-04	4	8
Transferase activity, transferring glycosyl groups	0.3769	3.58E-04	25	245
Carboxylic ester hydrolase activity	0.4342	4.12E-04	16	127
Organic cation transporter activity	0.4818	4.58E-04	7	30
Cytokine binding	0.5903	5.61E-04	15	118
Nonmembrane spanning protein tyrosine kinase activity	0.7624	7.24E-04	5	16
Oxidoreductase activity (acting on the CH-OH group of donors, NAD or NADP as acceptor)	0.8561	8.13E-04	16	135
Serine-type endopeptidase inhibitor activity	0.9624	9.14E-04	14	111
B. KEGG terms with E-values less than 1.0 within the sex-specific genes				
Tryptophan metabolism	4.31E-11	3.68E-13	21	61
Fatty acid metabolism	2.82E-06	2.41E-08	15	54
γ-Hexachlorocyclohexane degradation	2.81E-05	2.40E-07	12	40
Sulfur metabolism	0.0015	1.28E-05	5	8
Androgen and estrogen metabolism	0.0058	4.96E-05	8	29
Starch and sucrose metabolism	0.0135	1.16E-04	10	50
C21-Steroid hormone metabolism	0.0181	1.54E-04	5	12
Cytokine-cytokine receptor interaction	0.0221	1.89E-04	25	235
Tyrosine metabolism	0.0246	2.10E-04	8	35
Glyoxylate and dicarboxylate metabolism	0.0377	3.23E-04	4	8
Complement and coagulation cascades	0.0582	4.97E-04	11	70
Nicotinate and nicotinamide metabolism	0.1076	9.20E-04	8	43
Neuroactive ligand-receptor interaction	0.1083	9.25E-04	26	276
Methane metabolism	0.1583	0.0014	4	11
Jak-STAT signaling pathway	0.2517	0.0022	16	148
Nitrogen metabolism	0.4275	0.0037	4	14
C. Major gene families within the sex-specific genes				
Cytochrome P450		2.00E-12	25	82
Sulfotransferase		2.16E-05	6	12
Serine (or cystein) peptidase inhibitor		0.0026	10	63
Carbonic anhydrase		0.0105	4	16
Flavin monooxygenase		0.0116	3	9
ABC transporter		0.0234	7	51
Glutathione S-transferase		0.0376	4	23
Carboxyl esterase		0.0408	2	6
Solute carrier family		0.0987	24	322

Legend on next page.

other groups of genes, 5A and 5B, were sexually dimorphic in both WT and STAT5b-deficient mice and were unaffected by the loss of STAT5b. Three of the group 5A genes are Y linked (*Ddx3y, Eif2s3y, Jarid1d*) and, as a result, these genes show a high degree of male specificity in both WT and STAT5b-KO mice (Table 4). Two of these Y-chromosome genes are involved in transcription (*Jarid1d*) and RNA metabolism (*Ddx3y*) and could contribute to some of the STAT5b-independent sex differences exhibited by the other group 5A and 5B genes.

GH-activated STAT5b directly regulates several liver-expressed genes, including *SOCS2*, *IGFALS*, and *CIS*, each of which was found to be STAT5b dependent (supplemental Table 1). In the case of *IGF-I*, a 20–30% decrease in expression was seen in the absence of STAT5b in both males and females[1], in agreement with the approximately 30% decrease in plasma IGF-I levels seen in the same STAT5b-deficient mouse model (22) and in agreement with other studies pointing to a direct regulatory role by STAT5b (49–51). However, it seems unlikely that STAT5b directly regulates all 1715 of the genes presently found to be altered in expression in STAT5b-deficient mouse liver, many of which (987 of 1715; 58%) were apparently subject to negative regulation (Table 9). STAT5b can interact functionally with the corepressor silencing mediator of retinoid and thyroid hormone receptor, leading to repression of STAT5b-dependent transcription (52), and relief of such an inhibition may serve as a model for the apparent derepression of many female-specific genes presently seen in STAT5b-deficient male livers (Table 6). Examples of signaling cross-talk leading to negative regulation of gene transcription by STAT5b have been reported (53, 54); however, STAT5b is not known to exhibit strong or widespread repressor activity. The possibility that GH pulse-activated STAT5b may act in an indirect manner to regulate expression of at least some of the STAT5b-dependent genes is supported by a qPCR-based study of the temporal response of 15 sex-dependent liver genes, primarily *Cyps*, to continuous GH treatment (20). Three patterns of response to continuous GH were identified: early response (within 10 h), intermediate response (within days), and delayed response (not apparent till 7–14 d). The *Cyp* genes investigated were in the intermediate- and the delayed-response categories, suggesting that they are not directly regulated by GH and STAT5b, and raising the possibility that STAT5b may regulate sex-dependent *Cyp* expression indirectly, via the transactivation of early-response genes encoding transcriptional activators and repressors (20). For example, a STAT5b-dependent, male-specific transcriptional activator could contribute to the induced expression of group 1A genes, whereas a STAT5b-dependent, male-specific repressor could contribute to the apparent repression of group 1B genes.

Several such potential transcriptional regulators were identified in the present study, as were many receptors and genes involved in signal transduction, including various kinases and phosphatases (supplemental Tables 14–16). The sex- and STAT5b-dependent expression of these families of genes raises the possibility that a network of regulation may contribute to the large number of genes characterized by STAT5b dependence and sex-specific expression. Presumed primary target genes, such as the *CIS* gene, which inhibits GH-stimulated intracellular signaling and was presently found to require STAT5b for full expression, may modulate the activity of downstream targets by decreasing cell responsiveness to cytokine signaling. The temporal pattern of changes in gene expression in mice given a continuous infusion of exogenous GH (20), noted above, also suggests the involvement of multiple regulatory proteins in the sex-specific transcription of *Cyps* and other genes identified in the present study. Further studies will be required to evaluate the potential role of these factors as mediators of the effects of GH and STAT5b on sex-specific expression of *Cyps* and other genes in liver.

STAT5b disruption results in multiple physiological changes, including changes in body growth rates, circulating IGF-I levels, and perhaps plasma GH profiles (22), raising the possibility that these or other hormonal or metabolic changes could contribute to the observed global loss of male liver gene expression and sexual dimorphism. Thus, the liver RNA profiles of the global STAT5b-deficient mice characterized in the

[1] *IGF-1* is not included in our list of 2231 regulated genes because the average WT vs. STAT5b-KO expression ratios (1.27 in females and 1.42 in males) were below our threshold criteria of 1.5-fold. The correspondence between hepatic IGF-1 RNA suppression and the decrease in plasma IGF-1 levels seen in STAT5b-deficient male and female mice highlight the biological significance of changes in gene expression that are less than 1.5-fold.

Legend to Table 11. Genes meeting the criteria described in *Materials and Methods* for the M-WT:F-WT comparison were analyzed for enrichment of GO and KEGG terms within their descriptors. A total of 1374 genes define the subset that met the criteria and also had GO or KEGG descriptors. Terms with an E value less than 1.0 are shown for GO molecular function (A) and KEGG pathway (B). E values less than 1 indicate the term is likely to be enriched within the set of sex-specific genes rather than selected by chance. Also shown are the *P* value, number of genes within the subset containing the term (Overlap Gene Count), and the number of genes within the total set of all *Mus musculus* genes containing the term (Set Gene Count). A similar analysis was applied to gene symbol identifiers to identify gene family enrichment (C).

[a] The number of genes within the 1374 annotated, sex-specific genes that are annotated with the given category.

[b] The number of genes within all 28,489 mouse genes stored in Rosetta Resolver that are annotated with the given category.

present study could be due, in part, to changes in the pattern of pituitary GH secretion in these mice, *e.g*. as a consequence of the loss of STAT5b-mediated feedback inhibition of pituitary GH secretion in the hypothalamus (55). Such changes in GH feedback inhibition could lead to more frequent pituitary GH release and a female-like GH secretory pattern, which would, by itself, be sufficient to account for the widespread feminization of liver gene expression. This issue was addressed, in part, in an earlier investigation of the GH pulse responsiveness of STAT5b-deficient mice after hypophysectomy, which eliminates circulating GH and provided the opportunity to evaluate the liver's intrinsic responsiveness to plasma GH pulses applied exogenously and its dependence on hepatic STAT5b activity (56). The STAT5b-deficient hypophysectomized mice were found to be GH pulse resistant (20, 56), suggesting that the present requirement of STAT5b for sex-dependent liver gene expression is independent of any effect that *Stat5b* disruption may have on circulating hormone levels. Further confirmation of this conclusion will require characterization of mice with a liver-specific deficiency in STAT5b.

Future studies are required to determine which of the STAT5b-dependent genes described here may be directly activated by STAT5b in response to stimulation by male plasma GH pulses, and which genes may be activated or repressed as a result of a more complex regulatory network downstream from STAT5b. Further details regarding these STAT5b/GH-regulatory networks may be elucidated by monitoring the temporal patterns of change in sex-specific liver gene expression, *e.g*. in hypophysectomized mice given exogenous GH in a pulsatile pattern, to masculinize liver gene expression, or in intact male mice given a continuous infusion of GH, to feminize liver gene expression. It will also be of interest to determine what role, if any, STAT5a may play in sex-specific liver gene expression. STAT5a is closely related to STAT5b, and like STAT5b, exhibits sex-dependent responses to plasma GH stimulation. Moreover, STAT5a and STAT5b both appear to be required for the expression of certain GH-regulated Cyp steroid hydroxylases in female liver (15, 57). Studies on these sex-dependent and STAT5-regulated genes and the factors that govern the complex network of factors that are likely to be involved in the differential activation and repression of target genes between the sexes may provide important insight into the action of GH leading to differences in physiology between men and women, including sex- and GH pattern-dependent effects on human hepatic *CYP* expression and drug metabolism (58, 59).

MATERIALS AND METHODS

Animals

STAT5b-deficient mice, generated by insertion of a neomycin resistance cassette at the *Bam*H1 site interrupting codon 181, were described previously (22). Livers from 8- to 9-wk old WT and STAT5b-deficient mice, originally obtained from Dr. Helen Davey (Ag Research, New Zealand), were used in this study. Livers were harvested without regard to the plasma GH status of the individual mice at the time of killing. Livers were snap frozen in liquid nitrogen and stored at −80 C until use.

RNA Isolation

Total RNA was isolated from approximately 0.1 g frozen mouse liver using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. Twenty-four individual mouse liver RNAs (30 μg RNA per liver, dissolved in diethylpyrocarbonate-treated water) were used in this study: six WT male liver samples, six STAT5b-KO male livers, five WT female livers, and seven STAT5b-KO female livers. Three RNA pools were prepared for each group of livers, with each pool comprised of n = 2 individual liver RNA preparations (except as noted below) to help reduce variability in mRNA abundance between pools. One of the WT female liver RNA samples was included in two of the WT female pools, and three STAT5b-KO female RNA samples were pooled together to create one of the STAT5b-KO female RNA pools.

Data Generation and Acquisition

Expression levels for the liver RNA pools were determined using the approximately 23,500-feature mouse Mouse Oligo Microarray platform (Agilent Technology, Palo Alto, CA), which comprises a single 60-mer oligonucleotide for each gene. The actual number of genes analyzed is likely to be smaller than this number due to the presence of nonannotated sequences, some of which may duplicate results for other genes represented on the chip. The RNA pools (n = 3 for each of four sex-genotype combinations) were used in four separate competitive hybridization experiments in a loop design: male WT *vs.* female WT (M-WT:F-WT); male WT *vs.* male STAT5b-KO (M-WT:M-KO); female STAT5b-KO *vs.* female WT (F-KO:F-WT); and female STAT5b-KO *vs.* male STAT5b-KO (F-KO:M-KO). Each RNA pool was labeled with Cy3-dUTP or Cy5-dUTP in a reverse transcription reaction to generate fluorescent-labeled cDNA. The Cy3-labeled cDNA from one of the three male WT pools was mixed with the Cy5-labeled cDNA from one of the three female WT pools. The opposite-labeled cDNAs from both pools were also mixed. Together, these two mixed cDNA samples are considered a fluorescent reverse pair (dye swap) and were prepared for each of the four hybridization experiments. Two microarrays, one for each mixed cDNA sample, were hybridized for each fluorescent reverse pair. Three fluorescent reverse pairs, corresponding to the three pools of each liver RNA, were hybridized for each of the four microarray comparisons, giving a total of 24 microarrays. The fluorescence intensity values obtained from each microarray were normalized using Rosetta Resolver (Rosetta Biosoftware, Seattle, WA). The two halves of a single fluorescent reverse pair were averaged to remove any potential dye-dependent effects on the reported expression ratios. Expression ratios obtained in this study are included in supplemental Table 1. The data are also available for query or download from the Gene Expression Omnibus (GEO) web site at NCBI (http://www.ncbi.nlm.nih.gov/geo). Probe sequences are available upon request.

Statistical Analysis

A one-sample *t* test using GeneSpring 7.0 software (Agilent Technology) was applied to the $\log_2$ expression ratios for each gene. The *t* test implemented by the GeneSpring software package calculates the *P* value for the distribution of log

values as compared with a ratio of 1. A filter ($P < 0.05$) was applied to the *P* values to determine the statistical significance of each gene's differential expression for each of the four DNA microarray experiments (M-WT:F-WT, M-WT:M-KO, F-KO:F-WT, F-KO:M-KO). Multiple testing correction methods, such as Bonferroni or Holm step-down, were not applied to the *P* values because these options depend heavily on the independence of each gene's expression and thus filter out many *bona fide* regulated genes to avoid all type I errors; they are thus too restrictive in their effort to avoid false positives, as noted elsewhere (29). A differential expression filter (average ratio ≥ 1.5 or ≤ 0.66) was applied to the average gene expression values deemed statistically significant by the $P < 0.05$ filter. Threshold values of at least 1.5 or not more than 0.66 were chosen as described in our previous microarray study of sex specificity and GH-related rat liver gene expression (29). Genes for which at least one of the four average ratios passed the criteria for statistical significance and the differential expression threshold were included in our analyses (2231 genes in total; see *Results*).

The 2231 genes of interest were hierarchically clustered based on Pearson's correlation coefficient for the profile of the four average $\log_2$ ratios for each gene as implemented within GeneSpring. The experimental conditions were also clustered hierarchically, based on the profile of the average $\log_2$ ratios for the genes of interest for each array. The resulting gene tree and condition tree are presented in Fig. 1 (see *Results*). A system of binary and decimal flags was also established for clustering the genes based on expression ratios obtained in all four microarrays. For the purpose of this clustering, threshold ratios for differential expression were reduced to values of at least 1.25 or not more than 0.8 for the three arrays that involved STAT5b-KO liver RNAs, with retention of the $P < 0.05$ threshold for statistical significance. Average ratios meeting these threshold and significance criteria contributed to the binary- and decimal-based flag. Thus, genes with a M-WT:F-WT microarray ratio meeting the criteria were assigned a binary flag value of 1, whereas genes meeting the criteria for the M-WT:M-KO, F-KO:F-WT and F-KO:M-KO microarrays were assigned binary flag values of 2, 4, and 8, respectively. Genes not meeting these criteria were assigned flag values of 0. The sum of these binary-based flag values defines the whole number portion of the flag and was used as a simple method to identify which of the four microarrays met our criteria for inclusion for any given gene of interest, regardless of the direction (up or down) of the regulation. The flag value was then extended using decimal values of 0.1, 0.01, 0.001, and 0.0001, or 0.2, 0.02, 0.002, and 0.0002, for each of the four microarrays, to indicate the direction of regulation between the two conditions on the microarray. Thus, average ratios for the M-WT:F-WT microarray of at least 1.5 were assigned a decimal value of 0.2, to indicate up-regulation, whereas average ratios of at most 0.66 were assigned a value of 0.1, to indicate down-regulation. The three other microarray ratios were similarly flagged, based on threshold values of at least 1.25 or not more than 0.8, as indicated above, by advancing to a new decimal position for each microarray (*i.e*. the M-WT:M-KO flag is in the hundredths position, and so on). For each gene, the resulting binary sum describes which microarray ratios met the selection criteria, and the four-digit decimal value describes the direction of regulation (Total Flagging Sum). Data meeting the threshold criteria for two experimental conditions were analyzed by linear regression of the average $\log_2$ ratios (including the 95% prediction interval shown as *dashed lines* in Fig. 3 and supplemental Fig. 1) using GraphPad Prism version 4 (GraphPad Software, San Diego, CA).

Gene Annotation and Validation

The annotations for the 2231 genes of interest were validated using GenBank (NCBI). A BLAST search, using the 60-nucleotide probe sequences plated on the microarray, returned matching *Mus musculus* sequences contained in the nonredundant database. For each probe, the GenBank identification nos. (IDs) for returned sequences with identical matches over 56 or more nucleotides were retained and compared with the annotated GenBank ID. Of the 2231 probes, 1538 returned their annotated ID within the retained search results. GenBank ID annotations were not available for 297 of the 2231 probes. If no match for the GenBank ID was found within the BLAST search results, then the Entrez Gene (NCBI) family ID for the probe was compared with the Entrez Gene family ID for each of the retained search results. An additional 247 of the 2231 probes were validated by Entrez Gene family association between the probe and BLAST search results. Probes not validated using the BLAST search results were aligned to the GenBank sequence for their annotated GenBank ID using a semiglobal sequence alignment method. Of the remaining probes 88 aligned to their annotated sequence, despite the fact that it was not returned in the BLAST search results. Probes ($n = 61$) that did not align well to their annotated sequences had their annotations replaced with the gene to which they were found to be most similar. Genes were further characterized by general categories of cellular function based on known function or known functional domains within the encoded protein. The following 12 categories were used: cell adhesion, channel, cytoskeleton, metabolism, protein synthesis/metabolism, receptor, replication/apoptosis/chromosomal maintenance, secreted protein, signal transduction, trafficking, transcription, and transporter. Genes with no functional information, nonvalidated probes, or a domain structure suggestive of multiple functions were categorized as unknown.

GO and KEGG Term Enrichment Analysis

GO and KEGG molecular function annotation was available for a subset (1374 of 1603) of the sex-dependent genes that met our selection criteria (supplemental Table 1). These annotations were analyzed for term enrichment using Rosetta Resolver software. The GO term enrichment was based on a Considered Gene Count, which corresponds to the 28,489 genes in the mouse genome for which Resolver provided GO molecular function information. Because the GO term assigned to each gene represents a subclass of its parent class within the ontology, each gene was also assigned all parent GO class terms. For each of the 1053 GO categories present among the 1374 genes with GO or KEGG annotations, the number of genes assigned that term (Overlap Gene Count) was calculated along with a count of the Considered Genes (Set Gene Count). The probability for each category to be represented by at least the Overlap Gene Count within the 1374 genes, given the Set Gene Count among the Considered Genes, was calculated using the common hypergeometric distribution. An E value was calculated by multiplying the *P* value by the number of categories tested, 1053 GO categories and 117 KEGG categories, respectively. E values less than 1.0 represent categories that are unlikely to have been enriched by chance and are shown in Table 11. Gene family analysis was accomplished by searching the mouse genome for gene symbols using the family prefix (*e.g. Cyps* were counted using "cyp*[sym]" in a search of Gene on NCBI). The resulting number of family members was considered the Set Gene Count, and those family members also found in the 1603 sex-specific genes were considered the Overlap Gene Count. The *P* value was calculated in the same manner as the GO and KEGG enrichment analysis. E values were not calculated for these gene family analyses because the total number of genes to be tested could not be determined readily; instead, a *P* value threshold of 0.1 was used to select the gene families shown in Table 11C.

Acknowledgments

This paper is dedicated to the memory of Dr. Helen W. Davey (1957–2005), who originally provided the STAT5b-deficient livers used in the present study.

Received December 5, 2005. Accepted January 30, 2006.

Address all correspondence and requests for reprints to: David J. Waxman, Department of Biology, Boston University, 5 Cummington Street, Boston, Massachusetts 02215. E-mail: djw@bu.edu.

This work was supported by National Institutes of Health Grant DK33765 (to D.J.W.). K.H.C. received Training Core support from the Superfund Basic Research Center at Boston University, National Institutes of Health Grant 5 P42 ES07381.

Disclosure: K.H.C., M.G.H., S.H.P., and D.J.W. have nothing to declare. P.H. and W.J.R. are employed by and have equity interests in Merck and Co., Inc.

REFERENCES

1. Horvath CM 2000 STAT proteins and transcriptional responses to extracellular signals. Trends Biochem Sci 25:496–502
2. Darnell Jr JE 1997 STATs and gene regulation. Science 277:1630–1635
3. Grimley PM, Dong F, Rui H 1999 Stat5a and Stat5b: fraternal twins of signal transduction and transcriptional activation. Cytokine Growth Factor Rev 10:131–157
4. Frank SJ 2001 Growth hormone signalling and its regulation: preventing too much of a good thing. Growth Horm IGF Res 11:201–212
5. Lin JX, Leonard WJ 2000 The role of Stat5a and Stat5b in signaling by IL-2 family cytokines. Oncogene 19: 2566–2576
6. Herrington J, Carter-Su C 2001 Signaling pathways activated by the growth hormone receptor. Trends Endocrinol Metab 12:252–257
7. Veldhuis JD, Anderson SM, Shah N, Bray M, Vick T, Gentili A, Mulligan T, Johnson ML, Weltman A, Evans WS, Iranmanesh A 2001 Neurophysiological regulation and target-tissue impact of the pulsatile mode of growth hormone secretion in the human. Growth Horm IGF Res 11(Suppl A):S25–S37
8. Jansson JO, Eden S, Isaksson O 1985 Sexual dimorphism in the control of growth hormone secretion. Endocr Rev 6:128–150
9. Wiwi CA, Waxman DJ 2004 Role of hepatocyte nuclear factors in growth hormone-regulated, sexually dimorphic expression of liver cytochromes P450. Growth Factors 22:79–88
10. MacLeod JN, Pampori NA, Shapiro BH 1991 Sex differences in the ultradian pattern of plasma growth hormone concentrations in mice. J Endocrinol 131:395–399
11. Legraverend C, Mode A, Westin S, Strom A, Eguchi H, Zaphiropoulos PG, Gustafsson JA 1992 Transcriptional regulation of rat P-450 2C gene subfamily members by the sexually dimorphic pattern of growth hormone secretion. Mol Endocrinol 6:259–266
12. Sundseth SS, Alberta JA, Waxman DJ 1992 Sex-specific, growth hormone-regulated transcription of the cytochrome P450 2C11 and 2C12 genes. J Biol Chem 267:3907–3914
13. Waxman DJ, O'Connor C 16 March 2006 Growth hormone regulation of sex-dependent liver gene expression. Mol Endocrinol doi: 10.1210/me.2006-0007 (http://mend.endojournals.org/cgi/content/abstract/me.2006-0007v1)
14. Waxman DJ, Ram PA, Park SH, Choi HK 1995 Intermittent plasma growth hormone triggers tyrosine phosphorylation and nuclear translocation of a liver-expressed, Stat 5-related DNA binding protein. Proposed role as an intracellular regulator of male-specific liver gene transcription. J Biol Chem 270:13262–13270
15. Choi HK, Waxman DJ 1999 Growth hormone, but not prolactin, maintains, low-level activation of STAT5a and STAT5b in female rat liver. Endocrinology 140: 5126–5135
16. Choi HK, Waxman DJ 2000 Plasma growth hormone pulse activation of hepatic JAK-STAT5 signaling: developmental regulation and role in male-specific liver gene expression. Endocrinology 141:3245–3255
17. Tannenbaum GS, Choi HK, Gurd W, Waxman DJ 2001 Temporal relationship between the sexually dimorphic spontaneous GH secretory profiles and hepatic STAT5 activity. Endocrinology 142:4599–4606
18. Sueyoshi T, Yokomori N, Korach KS, Negishi M 1999 Developmental action of estrogen receptor-α feminizes the growth hormone-Stat5b pathway and expression of Cyp2a4 and Cyp2d9 genes in mouse liver. Mol Pharmacol 56:473–477
19. Waxman DJ 2000 Growth hormone pulse-activated STAT5 signalling: a unique regulatory mechanism governing sexual dimorphism of liver gene expression. Novartis Found Symp 227:61–74; discussion 75–81
20. Holloway MG, Laz EV, Waxman DJ 2006 Codependence of GH-responsive, sexually dimorphic hepatic gene expression on signal transducer and activator of transcription 5b and hepatic nuclear factor 4α. Mol Endocrinol 20:647–660
21. Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G, Ihle JN 1998 Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. Cell 93:841–850
22. Udy GB, Towers RP, Snell RG, Wilkins RJ, Park SH, Ram PA, Waxman DJ, Davey HW 1997 Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. Proc Natl Acad Sci USA 94: 7239–7244
23. Flores-Morales A, Stahlberg N, Tollet-Egnell P, Lundeberg J, Malek RL, Quackenbush J, Lee NH, Norstedt G 2001 Microarray analysis of the *in vivo* effects of hypophysectomy and growth hormone treatment on gene expression in the rat. Endocrinology 142:3163–3176
24. Gardmo C, Swerdlow H, Mode A 2002 Growth hormone regulation of rat liver gene expression assessed by SSH and microarray. Mol Cell Endocrinol 190:125–133
25. Lu C, Kasik J, Stephan DA, Yang S, Sperling MA, Menon RK 2001 Grtp1, a novel gene regulated by growth hormone. Endocrinology 142:4568–4571
26. Olsson B, Bohlooly YM, Brusehed O, Isaksson OG, Ahren B, Olofsson SO, Oscarsson J, Tornell J 2003 Bovine growth hormone-transgenic mice have major alterations in hepatic expression of metabolic genes. Am J Physiol Endocrinol Metab 285:E504–E511
27. Amador-Noguez D, Zimmerman J, Venable S, Darlington G 2005 Gender-specific alterations in gene expression and loss of liver sexual dimorphism in the long-lived Ames dwarf mice. Biochem Biophys Res Commun 332: 1086–1100
28. Amador-Noguez D, Yagi K, Venable S, Darlington G 2004 Gene expression profile of long-lived Ames dwarf mice and Little mice. Aging Cell 3:423–441
29. Ahluwalia A, Clodfelter KH, Waxman DJ 2004 Sexual dimorphism of rat liver gene expression: regulatory role of growth hormone revealed by deoxyribonucleic acid microarray analysis. Mol Endocrinol 18:747–760

30. Stahlberg N, Merino R, Hernandez LH, Fernandez-Perez L, Sandelin A, Engstrom P, Tollet-Egnell P, Lenhard B, Flores-Morales A 2005 Exploring hepatic hormone actions using a compilation of gene expression profiles. BMC Physiol 5:8
31. Thompson BJ, Shang CA, Waters MJ 2000 Identification of genes induced by growth hormone in rat liver using cDNA arrays. Endocrinology 141:4321–4324
32. Laz EV, Wiwi CA, Waxman DJ 2004 Sexual dimorphism of rat liver nuclear proteins: regulatory role of growth hormone. Mol Cell Proteomics 3:1170–1180
33. Shahan K, Denaro M, Gilmartin M, Shi Y, Derman E 1987 Expression of six mouse major urinary protein genes in the mammary, parotid, sublingual, submaxillary, and lachrymal glands and in the liver. Mol Cell Biol 7:1947–1954
34. Held WA, Gallagher JF, Hohman CM, Kuhn NJ, Sampsell BM, Hughes Jr RG 1987 Identification and characterization of functional genes encoding the mouse major urinary proteins. Mol Cell Biol 7:3705–3712
35. Clark AJ, Chave-Cox A, Ma X, Bishop JO 1985 Analysis of mouse major urinary protein genes: variation between the exonic sequences of group 1 genes and a comparison with an active gene out with group 1 both suggest that gene conversion has occurred between MUP genes. EMBO J 4:3167–3171
36. Wiwi CA, Gupte M, Waxman DJ 2004 Sexually dimorphic P450 gene expression in liver-specific hepatocyte nuclear factor 4α-deficient mice. Mol Endocrinol 18: 1975–1987
37. Lakso M, Masaki R, Noshiro M, Negishi M 1991 Structures and characterization of sex-specific mouse cytochrome P-450 genes as members within a large family. Duplication boundary and evolution. Eur J Biochem 195: 477–486
38. Akinola LA, Poutanen M, Vihko R 1996 Cloning of rat 17β-hydroxysteroid dehydrogenase type 2 and characterization of tissue distribution and catalytic activity of rat type 1 and type 2 enzymes. Endocrinology 137: 1572–1579
39. Crabb DW, Roepke J 1987 Loss of growth hormone-dependent characteristics of rat hepatocytes in culture. In Vitro Cell Dev Biol 23:303–307
40. Robertson JA, Haldosen LA, Wood TJ, Steed MK, Gustafsson JA 1990 Growth hormone pretranslationally regulates the sexually dimorphic expression of the prolactin receptor gene in rat liver. Mol Endocrinol 4:1235–1239
41. Klaassen CD, Liu L, Dunn II RT 1998 Regulation of sulfotransferase mRNA expression in male and female rats of various ages. Chem Biol Interact 109:299–313
42. McLellan LI, Hayes JD 1987 Sex-specific constitutive expression of the pre-neoplastic marker glutathione S-transferase, YfYf, in mouse liver. Biochem J 245:399–406
43. Heng YM, Kuo CS, Jones PS, Savory R, Schulz RM, Tomlinson SR, Gray TJ, Bell DR 1997 A novel murine P-450 gene, Cyp4a14, is part of a cluster of Cyp4a and Cyp4b, but not of CYP4F, genes in mouse and humans. Biochem J 325:741–749
44. Li-Hawkins J, Lund EG, Bronson AD, Russell DW 2000 Expression cloning of an oxysterol 7α-hydroxylase selective for 24-hydroxycholesterol. J Biol Chem 275: 16543–16549
45. Bergad PL, Shih HM, Towle HC, Schwarzenberg SJ, Berry SA 1995 Growth hormone induction of hepatic serine protease inhibitor 2.1 transcription is mediated by a Stat5-related factor binding synergistically to two γ-activated sites. J Biol Chem 270:24903–24910
46. Krebs CJ, Larkins LK, Price R, Tullis KM, Miller RD, Robins DM 2003 Regulator of sex-limitation (Rsl) encodes a pair of KRAB zinc-finger genes that control sexually dimorphic liver gene expression. Genes Dev 17:2664–2674
47. Tullis KM, Krebs CJ, Leung JY, Robins DM 2003 The regulator of sex-limitation gene, rsl, enforces male-specific liver gene expression by negative regulation. Endocrinology 144:1854–1860
48. Waxman DJ, Celenza JL 2003 Sexual dimorphism of hepatic gene expression: novel biological role of KRAB zinc finger repressors revealed. Genes Dev 17: 2607–2613
49. Davey HW, Xie T, McLachlan MJ, Wilkins RJ, Waxman DJ, Grattan DR 2001 STAT5b is required for GH-induced liver IGF-I gene expression. Endocrinology 142: 3836–3841
50. Woelfle J, Chia DJ, Rotwein P 2003 Mechanisms of growth hormone (GH) action. Identification of conserved Stat5 binding sites that mediate GH-induced insulin-like growth factor-I gene activation. J Biol Chem 278: 51261–51266
51. Woelfle J, Rotwein P 2004 In vivo regulation of growth hormone-stimulated gene transcription by STAT5b. Am J Physiol Endocrinol Metab 286:E393–E401
52. Nakajima H, Brindle PK, Handa M, Ihle JN 2001 Functional interaction of STAT5 and nuclear receptor co-repressor SMRT: implications in negative regulation of STAT5-dependent transcription. EMBO J 20:6836–6844
53. Luo G, Yu-Lee L 2000 Stat5b inhibits NFκB-mediated signaling. Mol Endocrinol 14:114–123
54. Zhou YC, Waxman DJ 1999 Cross-talk between janus kinase-signal transducer and activator of transcription (JAK-STAT) and peroxisome proliferator-activated receptor-α (PPARα) signaling pathways. Growth hormone inhibition of PPARα transcriptional activity mediated by stat5b. J Biol Chem 274:2672–2681
55. Bennett E, McGuinness L, Gevers EF, Thomas GB, Robinson IC, Davey HW, Luckman SM 2005 Hypothalamic STAT proteins: regulation of somatostatin neurones by growth hormone via STAT5b. J Neuroendocrinol 17: 186–194
56. Davey HW, Park SH, Grattan DR, McLachlan MJ, Waxman DJ 1999 STAT5b-deficient mice are growth hormone pulse-resistant. Role of STAT5b in sex-specific liver p450 expression. J Biol Chem 274:35331–35336
57. Park SH, Liu X, Hennighausen L, Davey HW, Waxman DJ 1999 Distinctive roles of STAT5a and STAT5b in sexual dimorphism of hepatic P450 gene expression. Impact of STAT5a gene disruption. J Biol Chem 274:7421–7430
58. Jaffe CA, Turgeon DK, Lown K, Demott-Friberg R, Watkins PB 2002 Growth hormone secretion pattern is an independent regulator of growth hormone actions in humans. Am J Physiol Endocrinol Metab 283: E1008–E1015
59. Cheung C, Yu A-M, Chen C-S, Krausz KW, Byrd LG, Feigenbaum L, Edwards RJ, Waxman DJ, Gonzalez FJ 2006 Growth hormone determines sexual dimorphism of hepatic cytochrome P450 3A4 expression in transgenic mice. J Pharmacol Exp Ther 316:1328–1334

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