Functional Avian Endocrinology
A Dawson & P J Sharp (Eds)

Transcriptional profiling in liver of hormonally-manipulated chickens

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Introduction

For decades, we have focused on the investigation of a single or, on occasion, multiple endocrine factors that regulate avian growth and development (8, 12). Much of our current understanding of the importance of a functional thyroid axis came from studies where thyroid-active substances were fed to young broiler chickens (12-14, 17). The metabolically-active thyroid hormone, T_3, plays a major role in maintenance of metabolic rate, body temperature and body composition (12) *via* paracrine adjustments in the insulin/glucagon (I/G) molar ratio (28). Short-term treatment of pre-market broiler chickens with a low level of dietary T_3 (0.25 ppm) reduces accumulation of excessive body fat and increases accretion of protein in skeletal muscle (12). On the other hand, the incorporation of a goitrogen [propylthiouracil (PTU)] into feed of young chickens leads to hypothyroidism, hyper-secretion of insulin and GH, retarded growth rate and mild obesity (12). The obesity of hypothyroid chickens is largely due to the absence of T_3 negative feedback on GH secretion (23) which in turn contributes to elevated insulin secretion and a higher I/G molar ratio.

Early studies in young broiler chickens showed that exogenous cGH fails to increase either growth rate or plasma IGF-I, albeit the accumulation of body fat was increased (7, 12, 15, 37, 39). This is unlike the typical mammalian response to exogenous GH which includes increased growth rate, elevated plasma IGF-I and a leaner body mass (11, 26, 40). Thus, slight obesity, elevated GH, higher I/G molar ratio, and down-regulation of the hepatic GH receptor (GHR) are common features of the GH resistance observed in young chickens (12, 36). The importance of a functional somatotropic axis is clearly revealed in the GHR-deficient dwarf chicken, which has a retarded growth rate, short

stature, mild obesity and hypothyroidism (17). Yet, the sex-linked dwarf chicken grows to two-thirds normal body weight, completely independent of GH action (16).

We have gained a more comprehensive view of endocrine regulation of growth and development from endocrine perturbation studies, where thyroid status was manipulated (*hypo- vs. hyper*-thyroidism) or where dietary T_3 was combined with exogenous growth hormone (cGH) (12). Hyperthyroidism (dietary T_3) reduces plasma GH levels and the I/G molar ratio, which lead to a marked depletion of body fat (12, 14). Decades ago, we discovered a very interesting synergism between two combined exogenous hormones – cGH + T_3 – which results in the dramatic depletion of body fat at market age (17).

The purpose of the present study was to use newly-developed chicken genomic tools (annotated cDNA microarrays, bioinformatic/ statistical tools, and a draft genome sequence) to examine gene expression patterns in liver in response to chronic treatment with thyroid-active substances (T_3 and PTU) alone, exogenous cGH alone, or GH combined with dietary T_3 (GH+T_3). This original microarray study has allowed the identification of several clusters of functional genes that respond to perturbations in the thyroid and GH axes, which ultimately interact to control growth and metabolism (19). With these transcriptional snapshots, we can begin to build functional maps of metabolic and regulatory pathways that regulate growth and development in the chicken – a model organism recently endowed with powerful tools of functional and structural genomics.

EST sequencing and development of liver-specific chicken cDNA microarrays

The first step toward advancing functional genomics in chickens was completion of a large international catalog of expressed sequences tags (ESTs) sequenced from numerous tissue cDNA libraries. We have constructed and normalized five single and multi-tissue chicken cDNA libraries for high throughput sequencing of ESTs and development of microarrays (19). About 37,500 ESTs were sequenced from chicken cDNA libraries which represent several important (immune, metabolic, somatic and neuroendocrine) physiological systems. The CAP3 sequence assembly program (32) was used to assemble contigs from all chicken ESTs in public collections (~409K ESTs) on March 1, 2003, including 37K EST sequences in the UD collection. Our CAP3 assembly of a chicken gene index (Version 2) revealed 31,472 contigs and 83,099 unassembled singlets ESTs. These contig (high-fidelity *in silico* cDNAs) and singlet sequences were then used in BlastX and BlastN analysis; the highest Blast hit score was used for annotation of the chicken ESTs.

Non-redundant sets of chicken ESTs (unigenes) were selected from our EST collection for PCR amplification of cDNA inserts and production of several chicken cDNA microarrays including the present 3.1K liver-specific array, an 8K metabolic systems array, a 6K neuroendocrine array, and the 14K Del-Mar Chicken Integrated Systems microarray (18). For the liver specific microarray, the non-redundant hepatic unigene set (3.1K) was selected from a total of 5,611 ESTs sequenced from the primary and normalized liver cDNA libraries (18, 19). The non-redundant liver-specific cDNAs were PCR amplified and robotically printed onto 8 x 12 cm nylon membranes in a 96-well format (36 spots/grid). Our CAP3 chicken EST database of assembled contigs and unassembled singlets is searchable by nucleotide sequence or keywords and gene lists are provided for each chicken microarray developed (http://udgenome.ags.udel.edu/~cogburn/).

Liver-specific microarrays and analysis of hepatic gene expression patterns

Total RNA (25 μg) was isolated from the liver of four birds per treatment group and reverse transcribed in the presence of ^{33}P-dCTP. The ^{33}P-labeled cDNA targets from each chicken (N=24) were hybridized overnight to individual liver-specific microarrays (Fig. 1). Images were captured overnight on a PhosphorImager screen and scanned by a PhosphorImager (Storm 840, Molecular Dynamics) into a tiff file. The tiff files were then imported into Pathways 4 software (Invitrogen) for determination of spot intensities and normalization (i.e., the spot intensity of each gene was normalized by dividing by the mean intensity of all spots on the microarray). The Pathways 4 software provides treatment contrast, a simple Student's *t*-test and fold-difference filters for analysis of microarrays. A subroutine was written for the Pathways 4 program to provide an output file of raw spot intensities which were used in our custom Bayesian analysis of miroarrays (BAM) program (18). Parameters of the stochastic model are first estimated by the maximum likelihood method (1). After estimating the model parameters, a Bayesian method (3) allows us to estimate the "real" gene expression level from rank normalized data, which minimizes the false discovery rate (41). The BAM model also accounts for systematic error in production of microarrays associated with the 36 cDNA source plates and the 96 pins used for printing the microarrays. The posterior probabilities are then used to identify differentially expressed genes.

Appropriate treatment contrasts were used to identify clusters of thyroid hormone (TH-) and/or GH-responsive genes. Genes were clustered according to two criteria: the significance of their spot intensities and fold-difference between treatments in the initial analysis

provided by the Pathways 4 program. For more robust statistical analysis, the significance level ($P<0.05$) was determined from Wilcoxon signed-rank analysis and the mixed-model BAM program (posterior probability >0.95). The data from the quantitative RT-PCR (TaqMan® assays) were analyzed using ANOVA and Wilcoxon rank sum test.

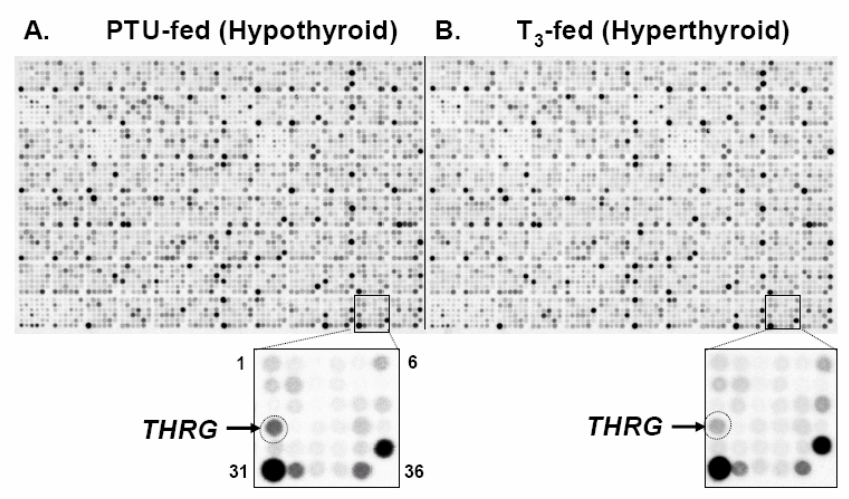

Fig. 1 Representative images of liver-specific chicken cDNA microarrays hybridized to liver RNA targets from a hypothyroid (PTU-treated) or a hyperthyroid (T_3-fed) broiler chicken. Liver total RNA (25 µg) was reverse transcribed in the presence of ^{33}P-dCTP and hybridized to a single membrane-based liver microarray. The 3,154 non-redundant cDNAs were robotically printed on a nylon membrane (8 x 12 cm) in a 96-well format (36 spots/grid). The images were developed on a PhosphorImager screen which was scanned into a Tiff-file by a PhosphorImager (Storm 840, Molecular Dynamics). The gene (spot) intensities were then analyzed with Pathways 4 software (Invitrogen). Insets show that the hepatic expression of an unknown EST is higher in the hypothyroid state (**A**) and lower in the hyperthyroid state (**B**). This unknown gene has been further characterized and named thyroid hormone repressible gene (*THRG*).

Manipulation of the thyroid and/or GH axis in young chickens

Four-week-old broiler cockerels (two pens of four birds/treatment) were fed thyroid-active substances for three weeks: propylthiouracil (PTU, 0.5%), T_3 (1.25 ppm) or given a daily *i.m.* injection of cGH (250 µg/kg) alone or daily cGH injection in combination with dietary T_3 (GH+T_3). Control feed (CF) was finely-ground commercial broiler-grower ration, whereas the identical ration was fed in pellet form to the buffer control (BC) and GH-injected groups. The average body weights of injected

birds (BC and GH treatments) fed the commercial ration (pellets) were slightly higher that the dietary treatment groups fed the pulverized ration (CF, PTU, T_3). The abdominal fat weight of PTU-treated birds was 53% higher ($P<0.05$) than that of the CF group, whereas dietary T_3 alone reduced abdominal fat by 52%, whereas the combined GH+T_3 treatment depleted fat by 77% (Fig. 2A). Plasma T_3 levels were reduced in hypothyroid chickens (67% reduction) and greatly elevated ($P<0.05$) in the hyperthyroid (T_3-fed) state (4-fold increase) (Fig. 2B). The further enhancement of plasma T_3 levels in GH+T_3-treated birds at 7 weeks of age represents an important synergism between the enhanced somatotropic and thyrotropic axes. The greatly elevated levels of T_3 in the GH+T_3 treatment would certainly support a higher metabolic rate and enhanced lipolysis. We suspect that the enhancement of plasma T_3 levels in the GH+T_3 treatment group was due to cGH depression of type 3 deiodinase (DI3) activity which degrades T_3 to metabolically inactive T_2 (see Van der Geyton *et al*., this volume). Earlier work has clearly shown that exogenous cGH elevates plasma T_3 levels by reducing DI3 activity in liver and kidney (21, 22). Thus, the combined treatment of GH+T_3 leads to a synergism between the thyrotropic and somatotropic axes and a remarkable depletion of body fat stores, which confirms our earlier studies (12).

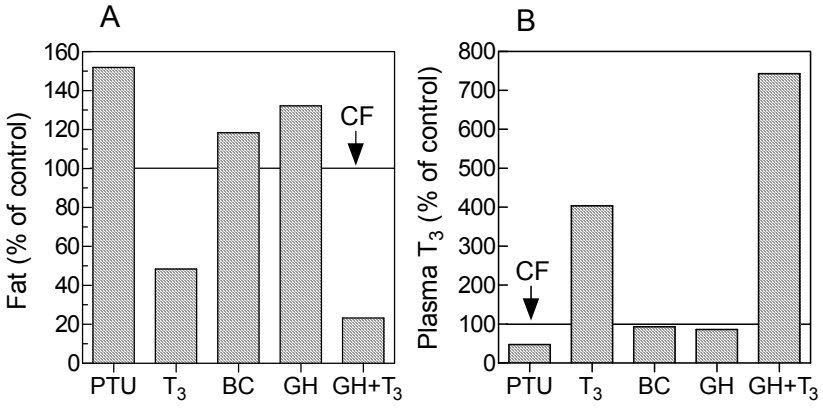

Fig. 2 Relative abdominal fat content (**A**) and plasma T_3 levels (**B**) of broiler chickens after chronic thyroid manipulation and/or cGH treatment. Birds were given either control feed (CF), 0.5% PTU, 1.25 ppm T_3 in ground feed or a single daily injection of buffer control (BC), 250 μg cGH/kg BW (GH) or GH+T_3 for three weeks (4 to 7 weeks of age). The data are presented as a percent of the CF group at 7 weeks of age (N=8 birds).

Transcriptional response to perturbation of the thyrotropic and somatotropic axes

Two representative images are shown after hybridization of liver-specific chicken cDNA microarrays with RNA isolated from liver of a hypothyroid (PTU-fed) (Fig. 1A) or a hyperthyroid (T_3-fed) (Fig. 1B) chicken. A total of 2606 clone pairs were filtered from this contrast (PTU *vs.* T_3) because their mean spot intensities were not significantly ($P<0.05$) different. The insets show the differential expression of an unknown EST, which we have named thyroid hormone repressive gene (*THRG*). *THRG* is expressed at higher levels in liver of hypothyroid (PTU-fed) chickens when compared to hyperthyroid (T_3-fed) chickens (Fig. 1B; Table 1). Gene cluster analysis, based on the differentially expression ($P<0.05$) and appropriate treatment contrast, was used to identify TH- and GH-responsive genes expressed in the liver (Fig. 3). Hypothyroidism (PTU *vs*. CF contrast) induced by dietary propyl-thiouracil (PTU) altered ($P<0.05$) expression of 165 liver genes (Fig. 3A). Within this cluster, a group of 144 genes was novel to PTU-induced hypothyroidism. The PTU-responsive genes include transcription factors (*Spot 14*, *C/EBPα*), several metabolic enzymes (*HMG CL, KHK, PGDS, BHMT),* transporters [*adipophilin (or ADFP), FABP*] and acute phase (*GSTα*, *CYP2C45, HSP90*) proteins.

Table 1 Hepatic genes with higher expression in *hypo*-thyroidism (PTU) or *hyper*-thyroidism (T_3)

Gene Name	**Microarray Fold-change**	**TaqMan® Fold-change**	**Cellular/ Molecular Function**
Genes up-regulated by *hypo*-thyroidism	(PTU/ T_3)		
Cyp2c45	+2.57		lipid metabolism
Glutathione S-transferase (GSTα)	+2.81	+8.4	detoxific-ation
Thyroid hormone repressible gene (THRG)	+2.11	+4.8	lipid metabolism
Acetyl-CoA acetyltransferase 2 (ACAT2)	+2.02		lipid metabolism
Adipophilin (ADFP)	+1.94	+9.7	lipid storage
Prostaglandin-D2 synthase (PGDS)	+1.92	+8.0	signal transduction
Longevity assurance homolog 2 (LASS2)	+1.70		cell growth
Polyamine oxidase (PAO)	+1.70		detoxific-ation

Catalase (CAT)	+1.65		peroxidase
Cysteine protease inhibitor (GHRG1)	+1.60		protein degradation
Sulfotransferase (SULT or GHRG2)	+1.62		xenobiotic metabolism
Angiopoietin-like 3 homolog (ANGPTL3)	+1.50		Lipid metabolism
Genes up-regulated by *hyper*-thyroidism	(T_3/PTU)		
Spot 14 (THRSPα)	+2.04	+2.2	transcription
Ketohexokinase β (KHK-β)	+1.80		carbohydrate metabolism
Hemopexin (*HPX*)	+1.73		heme transport
CCAAT/enhancer binding protein (C/EBPα)	+1.61	+1.6	transcription factor
Lactate dehydrogenase (LDH-β)	+1.57	+7.7	anaerobic glycolysis
Son of sevenless homolog 2 (SOS-2)	+1.54		apoptosis signaling
Adenylate cyclase (ADCY)	+1.50		signal transduction
Fatty acid synthase (FAS)	+1.50		lipogenesis
Glutamine synthase (GLNS)	+1.49		amino acid metabolism
Carboxypeptidase H (CPH)	+1.47		prohormone processing
Hydroxymethylglutaryl-CoA synthase (HMG CS)	+1.45		ketogenesis
Fused toes homolog (FTH)	+1.43		ubiquitin conjugation

Note: The genes in this table met both significance criteria for differential expression ($P<0.05$ and >1.4-fold). The expression of several genes was confirmed by a more sensitive independent method, quantitative RT-PCR or TaqMan® analysis.

On the other hand, hyperthyroidism (T_3 *vs.* CF contrast) induced by dietary T_3 led to the differential (up- or down-regulated) expression of 103 hepatic genes. This cluster of TH-responsive genes (51 were unique to T_3) includes *THRG, THRSP, LDHβ, HMG CS, ACAA*, *ApoA4*, *sulfotransferase* (or *GHRG2*), *IGF-I* and *GHR*. The largest cluster of thyroid-responsive genes was revealed by the extreme thyroid contrast (PTU *vs.* T_3), where 248 genes were differentially expressed (137 genes were unique to the hypo- *versus* hyper-thyroid contrast). A group of 70 common genes was found between the extreme thyroid contrast (PTU *vs.*

T_3) and the hypothyroid (PTU *vs.* CF) contrast. Only 12 genes were found in common between hypothyroid (PTU *vs.* CF) and hyperthyroid (T_3 *vs.* CF) contrasts. A group of 34 common genes was found between hyperthyroid (T_3 *vs.* CF) and the extreme thyroid (PTU *vs.* T_3) conditions.

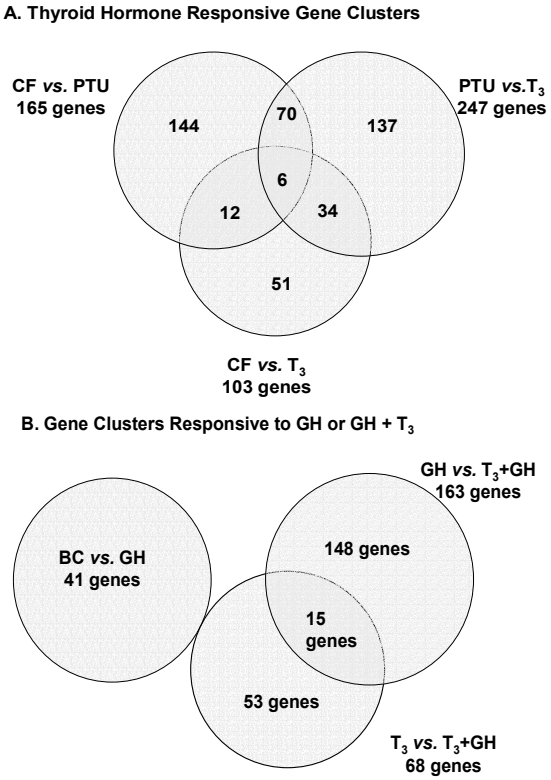

Fig. 3 Venn diagrams of hepatic gene clusters that respond to manipulation of the thyroid status (**A**) or to exogenous GH alone or GH combined with dietary T_3 (**B**). These gene clusters were derived from a Pathways 4 software analysis based on a single significance criterion (Student's *t*-test, $P<0.05$). Common genes shared between treatment contrasts are presented in the overlapping arcs.

Only 41 genes were differentially ($P<0.05$) expressed in the GH *vs.* BC contrast (Fig. 3B). A larger cluster of 166 genes was found in the GH *vs.* GH+T_3 treatment contrast. This cluster contains genes that are up-regulated by GH, where a group of 148 genes was unique to the combined GH+T_3 treatment. Similarly, only 68 differentially-expressed

genes were found in the T_3 *vs.* GH+T_3 contrast where a group of 53 genes was unique to the combined treatment. Fifteen differentially expressed genes were common between the GH *vs.* GH+T_3 contrast and the T_3 *vs.* GH+T_3 contrast.

Table 2. Hepatic genes that respond to exogenous GH alone *vs.* GH combined with dietary T_3

Gene Name	**Microarray Fold-change**	**Cellular/Molecular Function**
Genes up-regulated in GH *vs.* GH+T_3 contrast	**(GH/GH+T_3)**	
B-homocysteine methyltransferase (BHMT)	+1.8	methyltransferase activity
S-cyclophilin (S-CYLP)	+1.8	protein folding
F-box only protein 34 (FBXO34)	+1.7	unknown
Zinc finger protein 198 (ZNF198)	+1.6	signal transduction via FGF-R
β-Adaptin	+1.6	intracellular trafficking
Vesicle associated protein 1 (VAP1)	+1.6	intracellular trafficking
Acetyl-Coenzyme A acyltransferase 2 (*ACAA2*)	+1.6	□-oxidation of fatty acids
Transmembrane superfamily (TM4SF)	+1.5	cell development / growth
Sterol carrier protein 2 (SCP2)	+1.4	fatty acid transport
Recombination protein (REC14)	+1.4	signal transduction
TcD37 homolog (Prune)	+1.4	pyrophosphatase activity
Notch homolog 2 (NOTCH2)	+1.4	intercellular signaling
Genes up-regulated in GH+T_3 *vs.* GH contrast	**(GH+T_3/GH)**	
Lactate dehydrogenase-β (LDH-β)	+1.7	gluconeogenesis
Symplekin (SYMPK)	+1.7	mRNA cleavage/polyadenylation
Prothrombin (F2)	+1.7	blood coagulation
EGF-R substrate 8-like protein 2 (EPS8L2)	+1.7	signal transduction
Cytochrome P450 (Cyp2G1)	+1.7	oxidoreductase activity
Apoptosis-regulating basic protein (ARBP)	+1.4	unknown function
Hemopexin (HPX)	+1.4	heme transport
E3 protein (LAPTM5)	+1.4	hematopoiesis
Unknown EST	+1.4	unknown
Spot 14 (THRSPα)	+1.4	signal transduction

Note: The genes in this table meet both criteria for differential expression (P<0.05 and >1.4-fold).

A more stringent analysis based on two criteria, a significant *t*-test (P<0.05) and a >1.4-fold cutoff filter, was used to focus on major hepatic genes that respond to endocrine perturbation (Tables 1 and 2). Hypothyroidism induced by dietary propylthiouracil (PTU) up-regulated expression of 26 liver genes, including *Cyp2C45, GSTα, THRG*, *adipophilin* (or adipocyte differentiation factor protein, *ADFP*), *ACAT2, PGDS, BHMT, catalase* (*CAT*), longevity assurance homolog 2 (*LASS2*), and two GH-regulated genes [cysteine protease inhibitor (*GHRG1*) and sulfotransferase (*GHRG2*)] (Table 1). A cluster of 46 genes was up-regulated [i.e., a higher T_3/PTU ratio] by hyper-thyroidism; these genes include transcription factors (*Spot 14*, *C/EBPα*), signaling factors *(SOS-2, ADCY*), several metabolic enzymes (*LDHβ, KHK-β, FAS, GLNS, CPH, HMG CS*), and transport proteins (*HPX, L-FABP* and *FTH*).

GH treatment alone (GH *vs.* BC) yielded only five differentially expressed genes (*HSP73, Cyp2H1, V-ATPase, U2AF35 and Gal NAc4ST-2*) that met the more stringent significance criteria (P<0.05 and >1.4-fold difference). Similarly, only 25 genes in the GH *vs.* GH+T_3 contrast met the more stringent criteria (Table 2). The genes with higher expression in the GH alone *vs.* GH+T3 contrast included several enzymes (*BHMT, ACAA2* and *Prune*), signal transduction factors [*Zinc finger protein 198* (*ZNF198*), *REC14* and *NOTCH2*] and signaling/transport proteins (*β-Adaptin, VAP1, TM4SF, SCP2*). In addition, several genes were expressed at higher levels in the GH+T_3 *vs.* GH contrast [*LDH-β, SYMPK, prothrombin* (*F2), Cyp2G1, ARBP, HPX,* and *THRSPα*]. The expression of some of these genes was also increased in the hyperthyroid (T_3-fed) state (Table 1).

The transcriptional response of 14 genes (Table 1) was verified by a more sensitive independent method, quantitative RT-PCR (TaqMan®) analysis (Fig. 4). The hepatic expression of *GSTα* (Fig. 4A) and *PGDS* (Fig. 4B) was 8-fold higher (P<0.05) in PTU-fed hypothyroid chickens when compared to other treatments. The hepatic expression of the novel thyroid hormone repressible gene (*THRG*) was depressed 5-fold by T_3 alone or when T_3 was combined with GH (Fig. 4C). Also, the abundance of *THRG* is depressed by synthetic fatty acids and increased by either dexamethazone (10) or prolonged fasting (18). On the other hand, dietary T_3 alone or T_3+GH greatly increased (7- to 8-fold) the hepatic expression of *LDH-β,* a key enzyme in the gluconeogenic/ glycolytic pathway which reversibly converts lactate to pyruvate. Thus, these four genes and most of those listed in Table 1 appear to respond to manipulation of thyroid status. The T_3-regulated genes encompass a wide range of cellular functions: transcription (*THRSPα, C/EBP, PPARγ*),

metabolism (*ACAT2, LDH-β, HMG CS, ADFP*), signal transduction (*GHR, PDGS*), protein turnover (*GSTβ, BHMT*), intracellular trafficking, etc. Several of the T_3-responsive genes identified in this experiment with chickens (Table 1) were also induced in liver of hypothyroid rats given an acute injection of T_3 (43).

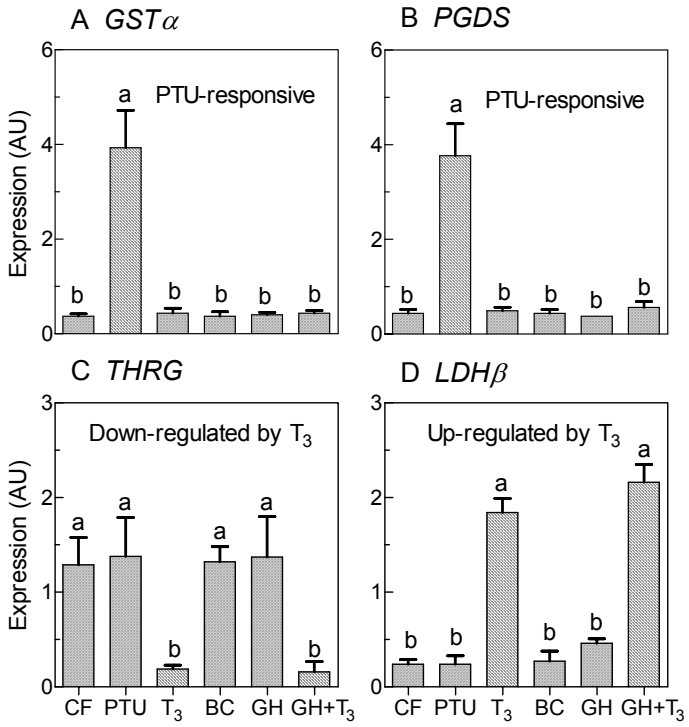

Fig. 4 Thyroid hormone-responsive gene expression verified by TaqMan analysis. The same total RNA samples used for microarray analysis were also used for quantitative RT-PCR (TaqMan) analysis. Each value [in arbitrary expression units (AU)] represents the mean±SEM of four birds/treatment group, where means with different superscripts differ significantly ($P<0.05$).

Elevation of *GSTα* and *CYP2C45* (2) could be a consequence of detoxification of PTU rather than a response to hypothyroidism. However, GST and several cytochromes involved in detoxification and xenobiotic metabolism were also induced by chronic GH treatment in hypox rats (24). Glutathione S-transferase (*GSTα*) catalyzes the conjugation of glutathione to electrophilic compounds, which protects cellular macromolecules from xenobiotics (31). In liver, GSTα exhibits

glutathione peroxidase activity which protects cells from reactive oxygen species. Glutathione-independent PGDS is a thyroid-hormone responsive gene (25) that uses GSH as a cofactor to catalyze the isomerization of PGH_2 (an intermediate) into PGD_2 Prostaglandin D_2 and its derivative (PGJ2) are potent activating ligands of peroxisome proliferator-activated receptors (*PPARα, -β* and *-γ* (44). The PPARs are transcription factors that belong to the nuclear receptor superfamily which controls expression of numerous metabolic enzymes (4). In our study, the expression of PPARγ was elevated by 4-fold in PTU-fed chickens (data not shown). Activated PPARs enhance the transcription of adipophilin (*ADFP*) (see Fig.5C) (6, 27), while ADFP is a lipid droplet component and a reliable indicator of lipid load in numerous cells (29, 33). The present microarray study shows that the abundance of *PGDS* and *ADFP* (Fig. 4B) is elevated in the hypothyroid (PTU-fed) condition which promotes fat deposition in the chicken (Fig. 2A).

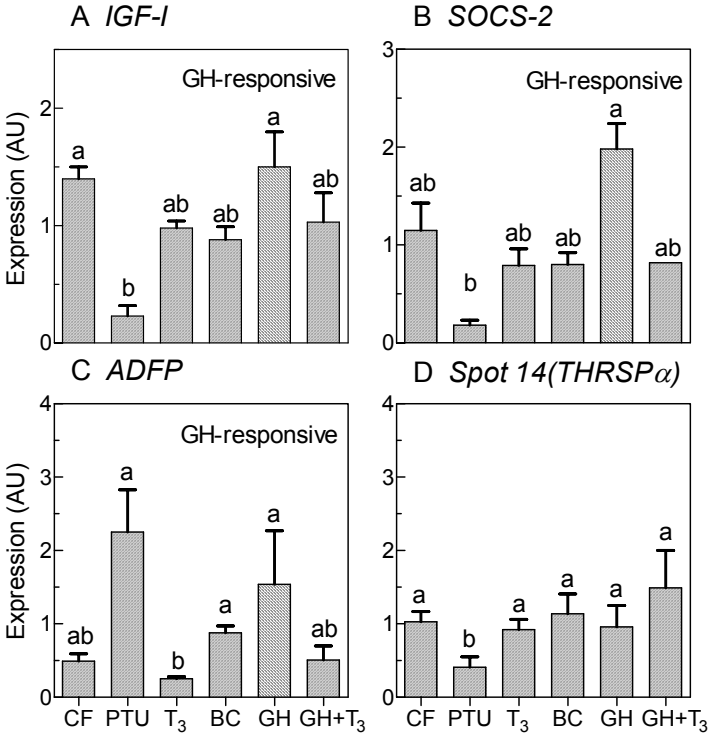

Fig. 5 Growth hormone-responsive gene expression verified by TaqMan analysis These four genes are also responsive to PTU-induced hypothyroidism. The same total RNA samples used for microarray analysis were also used for quantitative RT-PCR (TaqMan) analysis. Each

value (arbitrary expression units, AU) represents the mean±SEM of four birds/treatment group, where means with different superscripts differ significantly ($P<0.05$).

The expression of some GH-responsive genes was confirmed by TaqMan analysis (Fig. 5). Hepatic levels of both *GHR* (not shown) and *IGF-I* (Fig. 5A) mRNA were depressed in hypothyroid chickens. The effect of hypothyroidism on hepatic *IGF-I* expression is also reflected by depressed plasma IGF-I levels (14). In the present study, daily GH injection enhanced expression of both *IGF-I* (Fig. 5A) and suppressor of cytokine signaling 2 (SOCS2) (Fig. 5B). Hepatic abundance of *ADFP* was increased by GH injection or dietary PTU, which also elevates plasma GH levels (Fig. 5C). The expression of *FABP* (not shown) and *THRSPα* (*Spot 14*) (Fig. 5 D) are also depressed in the (PTU-induced) hypothyroid state. The expression of *THRSPα* was highest in birds given the GH+T_3 treatment combination. The abundance of *GHR* and *IGF-I* mRNAs in liver is depressed by PTU, whereas PTU-induced hypothyroidism increases hepatic expression of catalase (*CAT*) and longevity assurance homolog 2 (*LASS2*) (Table 2). These genes are involved in GH-GHR-IGF-I signal transduction pathway; they also belong to the "longevity" pathway (www.biocarta.com) which focuses on the IGF-I receptor as a mediator of increased lifespan in calorie-restricted animals (30).

Mapping of functional metabolic pathways in chickens

We have used existing models of metabolic and regulatory pathways in other organisms to build working models of metabolic and regulatory pathways that are specific to the chicken (18, 19). The transcriptional responses to thyroid perturbation were used to build a working model of thyroid control over several metabolic processes (Fig. 6). Many of the same genes presently identified as TH- and/or GH-responsive genes also appear to respond to other metabolic perturbations (18). Ligand-activated thyroid hormone (T_3) receptors (*TRα* and *TRβ*) belong to the nuclear receptor superfamily of ligand-activated transcription factors (*TR, RXR, PPAR, COUP-TF, THRSP)* that control several metabolic pathways. Orphan receptors are nuclear receptors for which the ligand is not yet known. Steroids, vitamins, thyroid hormones, retinoids, and some metabolites (glucose, sterols) serve as activating ligands for formation of homo- (i.e., *THRSP-THRSP*) or hetero-dimers (i.e., T_3-TR-RXR) of transcription factors.

Spot 14 (or *THRSP)* is an important transcription factor that controls expression of major metabolic enzymes in response to a number of perturbations (developmental, nutritional and hormonal). The transcription of *THRSP* is activated by ligand-activated receptors (T_3-*TR*,

PGJ_2-PPARγ, COUPTF) which form homo- and hetero-dimers and serve as activators of transcription (Fig. 6). The *THRSP* promoter region contains three thyroid response elements (TREs) that work

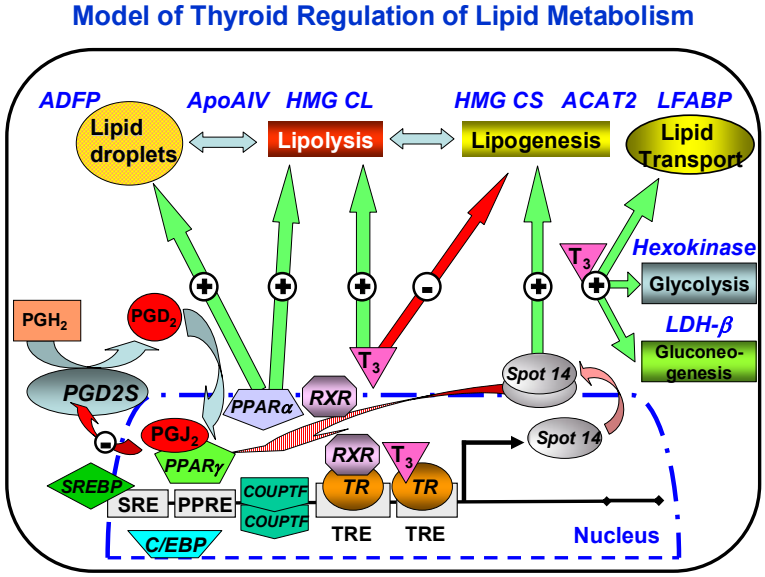

Fig. 6 Working model of thyroid regulation of energy metabolism in chicken liver. This diagram shows some regulatory factors that interact with the *Spot 14* (*THRSP*) promoter region and control its transcription. Thyroid hormone (T_3) interacts with one of its nuclear receptors (*TRα* or *TRβ*) to exert its influence on transcription of genes that maintain energy homeostasis. An example of a thyroid-responsive gene is presented above each metabolic process. The TRs belong to the ligand-inducible transcriptional activators in the nuclear receptor superfamily (*THRSPs, PPARs, COUPTF, SREBP, C/EBP*) whose ligands [steroids, thyroid hormone (T_3), metabolites, vitamins, prostaglandins (PGJ_2)] activate gene transcription. The ligand (T_3)-activated transcription factor (TR) binds to its response element (TRE) in the promoter region of target genes (*THRSP*). Transcription of duplicated chicken *Spot 14* genes (*THRSPα* and *–β*) is controlled by other members of the nuclear receptor superfamily (*TRs, COUPTF, PPARs, RXR*). Ligand-induced transcription factors form homo- and hetero-dimers which bind to their response elements (SRE, PPRE) on promoters and activate transcription of genes that regulate growth and metabolism.

synergistically and interact with far upstream region (FUR) elements to maximize T_3 responses in cultured hepatocytes (38). Apparently, the human *THRSP* promoter responds more robustly to T_3 than glucose,

while the rat *THRSP* promoter region is more responsive to glucose than T_3 (9). Homodimers of *THRSP* interact with and activate chicken ovalbumin upstream promoter-transcription factor 1 (COUP-TF1) in promoting transcription of L-type pyruvate kinase (*L-PK*) through an interaction with specificity protein 1 (Sp1) (20). Furthermore, the mammalian *THRSP* promoter contains multiple response elements that respond to thyroid hormone (TRE) (39), carbohydrates (ChoRE) (35) and sterols (SRE, sterol response element), particularly SREBP1c (34). Thus, multiple response elements exert *THRSP*'s control over the expression of key lipogenic, glycolytic and gluconeogenic enzymes in a tissue-specific and fuel-dependent manner (5). We have discovered and characterized duplicated Spot 14 genes (*THRSP*α and β paralogs) in the chicken (42). A unique insertion/deletion (indel) polymorphism was found in each paralog near the DNA binding motif. These *THRSP* polymorphisms could certainly increase the complexity of transcriptional responses to homeo- and hetero-dimers of this transcription factor. Furthermore, the polymorphic *THRSP* genes are located in quantitative trait loci for fatness on the long arm of GGA1 in a fast-growing x slow-growing F_2 resource population. Therefore, *THRSP* appears to play a key role in regulating the expression of several lipogenic enzymes in the chicken [see Fig. 4 in (19)].

Summary

We have developed several tissue-specific and systems-wide chicken cDNA microarrays for analysis of multiple transcriptional snapshots under various perturbations. Our current interest lies in unraveling the gene networks controlling growth and development. Altered gene expression patterns reflect changes in metabolism, transport and storage of nutrients in response to manipulation of the thyroid and/or GH axes. It is also clear that normal growth and development is regulated by complex gene interactions between the thyrotropic and somatotropic axes which have not been clearly defined. These initial studies demonstrate the power of DNA microarrays for transcriptional profiling and discovery of key regulatory genes. With this new information, we have begun to build functional maps of the metabolic and regulatory pathways that regulate important growth traits in the chicken.

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