Review Article

Structural Features and Transcriptional Activity of Chicken PPARs (α, β, and γ)

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While an understanding of lipid metabolism in chickens is critical for a further improvement of food production, there are few studies concerning differences in lipid metabolism mechanisms between chickens and other species at a molecular level. Chickens have three PPAR gene subtypes (α, β, and γ) that function differently from those present in humans and mice. The chicken PPAR-gamma (cPPARγ) gene is shorter than that in humans and lacks a γ2 isoform. Moreover, in serum-free media, cPPARγ shows high transcriptional activity without exogenous ligands. Luciferase reporter assays were used to examine the effect of sera on cPPAR transcriptional activities and showed that adult bovine serum and chicken serum highly activate cPPARα and β functions. Moreover, we found that bezafibrate induces the transactivation function of cPPARβ, but not human PPARδ (human PPARβ ortholog). This ligand selectivity relies on one amino acid residue (chicken: Val419, human: Met444). These results show the possibilities for unique functions of cPPARs on chicken-specific lipid glucose metabolism. As such, a better understanding of the molecular mechanisms of lipid metabolism in chickens could result in higher productivity for the poultry industry.

1. Genomic Organization of Chicken PPARs (cPPARs)

The mechanisms of lipid and glucose metabolism for energy storage and homeostasis differ between species in ways that are not fully understood. To improve food production, comprehending the molecular basis for lipid and glucose metabolism in domestic animals is a significant issue. In avian species, lipids, especially triglycerides, are thought to be stored in adipocytes, hepatocytes, and growing oocytes. Excessive accumulation of lipids in the adipose tissue of broilers is a major concern for producers, because excessive fat deposits result in lower meat yields.

In 2000, we reported the cloning of three chicken PPAR subtype genes (α, β, and γ) from cDNA libraries prepared from 2.5-day-old chicken embryos and adult adipose tissue [1]. For cPPARγ, amino acid residues predicted for phosphorylation by MAP kinase [2] (Ser82 in cPPARγ) and Cdk5 [3] (Ser243 in cPPARγ) were conserved between mammals and chicken PPARγ. Moreover, predicted SUMOylation target residues (Lys77 and Lys365 in cPPARγ) were also conserved in cPPARγ [4]. This shows the possibility that the transcriptional activity of cPPARγ regulates growth factors and that cPPARγ activation has an anti-inflammatory effect. However, we were unable to isolate the PPARγ2 splicing variant identified in mammals [5, 6]. This variant is likely absent in chickens because Sato et al. also could not isolate chicken PPARγ2 by 5′-RACE [7] and because no sequence similar to a PPARγ2 isoform has been found in the chicken genome database (Figure 1). The PPARγ2 isoform has not been reported in Xenopus and zebrafish either. Furthermore, while the structural organization of PPARα and β/δ genes is largely conserved from humans to chickens, the PPARγ isoform is not. The cPPARγ gene (Ensembl ID: ENSGALG00000004974) has 6 exons and extends across ~50 kilobases (kb) of genomic DNA (Figure 1, upper panel). In contrast, the human PPARγ gene (Ensembl ID: ENSG00000132170) consists of 8 and 7 exons for the γ1
and γ2 subtypes, respectively, and extends across more than 160 kb of genomic DNA (Figure 1, lower panel). Other species such as squirrel (Ensembl ID: ENSSTOG0000012778), hedgehog (ENSEEU00000363), Chinese softshell turtle (ENSPSIG0000011100), and Anole lizard (ENSC- AG0000013360) have the chicken-type organization of PPARγ genes.

In mammals, several polymorphisms of PPAR genes have been associated with metabolic disorders. In chicken, single nucleotide polymorphism (SNP) analysis has been conducted to find the association with lean and fat broilers [8]. In this study, PPARγ c-75G>A is reported to be associated with abdominal fat weight and also correlates with SNPs of other lipid metabolism-related genes (Fsbp4, C/ebpα, etc.).

Promoter analysis of the cPPARγ gene revealed that transcription factor binding sites (such as C/EBPα, Sp1 and AP1) exist as is the case with mammalian PPARγ1 and PPARγ2 promoters. Interestingly, structure of cPPARγ promoter is more similar to the mammalian PPARγ2 promoters compared to PPARγ1 promoters [9]. These results suggest that C/EBPα-PPARγ pathway is conserved across the species and that PPARγ2 gene first appeared in the course of evolution when additional PPARγ isoforms have been acquired.

2. Expression Patterns of cPPAR mRNAs

The mRNA expression patterns of cPPARs are similar to rodents and primates. In adult chicken, cPPARα is highly expressed in the heart, liver, and kidney [10]. As is the case for PPARβ or δ in other species, PPARβ is expressed ubiquitously. Meanwhile, high expression of cPPARγ is observed in fat tissue as well as the kidney, heart, brain, liver, and intestine [7, 11]. Recent studies using renal-specific PPARγ knockout mice have shown that the gene regulates body weight by controlling water channel activities [12]. Thus, cPPARγ may also regulate body weight in chickens.

Hojo et al. examined cPPAR mRNA expression patterns in digestive organs during embryonic development in chickens [13]. In embryonic day 6 (E6), E9, and E12 embryos, PPARα is expressed in the epithelium, smooth muscle, and small intestine. PPARβ is expressed in the epithelium and moderately in mesenchymal tissue on E6, E9, and E12. PPARγ is weakly expressed in the epithelium on E9 and E12 and expressed in the medial and basal parts of the small intestine villi, the villi of PH3 and 14, and the crypt epithelium of the large intestine.

3. Regulators of cPPAR mRNAs

PPAR mRNA levels are known to be regulated in aging, metabolic disorder, and cancer [14]. In general, mRNA levels of PPARs or their target genes are upregulated in obesity because of increased fatty acids. Several regulators of PPAR mRNAs have recently been identified in chicken.

In adipocyte, accumulation of nonesterified fatty acids (NEFA) induces cPPARγ mRNA, and a cPPARγ downstream gene (adipocyte fatty acid binding protein (A-FABP)) controls the concentration of lipid and NEFA [15]. In fatty broiler chickens, cPPARγ mRNA expression is induced in liver but not in adipose [16]. Because PPARγ mRNA is induced in adipose tissue of obese mammals [17, 18], the result indicates a divergence of PPAR signal transduction mechanisms between avians and mammals.

Regulation of cPPARα mRNA is also reported. Comparison of gene expression profiles in the intramuscular fat of two broiler lines showed that PPARα and MAPK pathway are enhanced in both Beijing-you (BJY), which is a slow-growing Chinese breed possessing high meat quality, and Arbor Acres (AA), a commercial fast-growing broiler line [19]. Upon feed restriction, mRNA expression levels of cPPARα and one of its target gene, peptide transporter 1 (PepT1), are induced in the jejunum [20].

In myoblasts, isoproterenol (a β-adrenergic receptor agonist) and fatty acid enhance the phosphorylation of cPPARα. The mRNA of UCP3 is induced through cPPARα and AMPK-PKA pathway [21]. In spleen, cPPARα and γ mRNAs are induced by lutein, a plant carotenoid, and reduced by lipopolysaccharide (LPS) [22]. These results indicate that some regulators of cPPARs mRNA expression are conserved between chicken and mammals.

4. Comparison of the Amino Acid Sequences between Chicken and Mammalian PPARs

The amino acid sequence in the DNA binding domain (DBD) of cPPARs shows high similarity with mammals (cPPARα versus hPPARα: 95%; cPPARβ versus hPPARδ: 98%; cPPARγ versus hPPARγ: 100%). The similarity of the ligand binding domain (LBD) is also high for PPARα and PPARγ (cPPARα versus hPPARα: 96%; cPPARγ versus hPPARγ: 96%). However, the LBD of cPPARβ shows lower similarity between chicken and humans (90%), mice (86%), and Xenopus (78%). Because this diversity implies different ligand responses, we conducted luciferase reporter assays to examine the ligand-inducible transcriptional activity of cPPARs.

5. Transcriptional Activities of cPPARs

We first generated cDNA expression vectors of cPPAR LBDs fused to GAL4 DBD. The ligand-inducible transcriptional
Although distinct endogenous ligands for chicken PPARs have not yet been revealed, fatty acids and their derivatives are strong candidates.

6. Bezafibrate Is a Ligand for Avian and Xenopus PPARβ

We next examined the effect of known PPAR activators (including fibrates and thiazolidinediones) on the transactivation function of cPPARs. Bezafibrate, a known ligand for Xenopus PPARβ (xPPARβ) [25], enhanced the ligand-inducible transactivation function of xPPARβ in our hands (data not shown) and cPPARβ, but not hPPARδ [1].

This result shows that bezafibrate selectively activates cPPARβ despite the relatively high structural similarity between cPPARβ and hPPARδ LBDs. We looked for the amino acids that are conserved between human and mouse PPARδ, but not chicken PPARβ and found that 15 amino acid residues were altered between chicken and mammals (Figure 4(a)). To identify the amino acid residue(s) critical for bezafibrate-dependent activation, we generated cDNA expression vectors carrying point mutations of relevant amino acid residues [23].

We next investigated the effect of serum on the transcriptional activities of cPPARs. It was found that adult bovine serum induces cPPARα transcriptional activities to a greater extent than fetal bovine serum and calf serum (Figure 3(a)). Interestingly, chicken serum (ChS) highly potentiated the transcriptional activities of cPPARα and β as compared to bovine serum. ChS also activated the transcription function of hPPARs (Figure 3(b)). Furthermore, using lipid extracts from chicken serum by a previously established method [24], we showed that the lipid extracts retain the enhancement effects of chicken serum for the transcriptional activities of cPPARα and β (Figure 3(c)).

These results show that serum from adult animals (chicken and bovine) contains more endogenous PPARβ ligands than that from fetal or young animals (bovine). Although the identity of PPARβ ligand(s) in adult serum has been unclear, fatty acids and their derivatives are strong candidates.
Figure 3: Effect of serum on the transactivation function of cPPARs. (a), (b). Effects of serum on the transactivation function of PPARs. Serum-induced transactivation function of cPPARs (a) or hPPARs (b). After transfection with each plasmid mixture, cells were cultured with indicated serum conditions for 36 hrs, then lysed, and luciferase reporter assays were performed as described previously [1]. FBS: fetal bovine serum, CBS: calf bovine serum, BS: adult bovine serum, and ChS: chicken serum. (c) The effect of lipid extract from chicken serum (ChS) on the transactivation function of cPPARs. After being transfected with each plasmid mixture, cells were cultured for 36 hrs in DMEM/10% FBS with or without 5% ChS or ChS lipid extract equivalent of 2% ChS. Then cells were lysed and luciferase assays were performed. Data are derived from triplicate points from two independent experiments and represented as the mean ± SE; n = 6.
acid change may reflect the difference between chicken and mammals in the regulation of energy storage [26].

Moreover, identification of amino acid residues involved in ligand interactions will help to generate PPARδ-specific ligands and facilitate the development of drugs to treat metabolic syndromes. Thus, investigation of species-selective PPAR ligand activity is significant for understanding species-specific lipid metabolism and development of new drugs.

7. Effects of Fibrate on Hens

There are few in vivo analyses of chickens fed with PPAR ligands. A previous report showed that hens treated with clofibrate (cPPARα ligand) had lower food intake and lost body weight during the 5-week feeding period, which in turn reduced egg production due to the absence of large follicle(s) and the presence of few small yellow and white follicles in the ovary [27]. Moreover, triglyceride levels in the livers of clofibrate-treated hens were reduced and mRNA levels of known PPARα target genes such as Acyl-CoA oxidase (ACO), hepatic lipase, and lipoprotein lipase (LPL) were induced. In contrast, mRNAs of insulin-induced genes (Insig-1, SREBP-2, FAS, HMG-CoA reductase, and LDL receptor) were downregulated by clofibrate treatment. These results show that, as with mice and humans, cPPARα regulates lipid metabolism in the liver and controls appetite [28].

8. Conclusions

In this paper we discussed the properties of chicken PPARs based on their genomic structural organization and ligand-inducible and -independent transactivation functions. Evolutionary changes in the PPARγ genomic structure may be one critical factor for species-dependent lipid homeostasis. Because there are few reports concerning the promoter activity of PPARγ gene in chickens and other nonmammals, more detailed analyses will be required. Interestingly, the ligand-inducible transcriptional activities of cPPARs do differ from mammalian PPARs. Because a single amino acid change leads to a selective ligand response, the spectrum of endogenous ligands of PPARs in avian species may be different from that of mammals. As such, further analysis will be required to understand the comparative endocrinology and this information will be useful for industrial applications in the poultry and other food industries.

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