原著論文

The promoter activity of the chicken liver receptor homolog-1 (LRH-1) gene in chicken ovary-derived (COV1) cells

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Abstract: LRH-1 is expressed in the steroidogenic organs and shares a role in steroidogenesis with SF-1, which is a paralog of LRH-1. We previously reported that the chicken LRH-1 gene is transcribed from at least five different transcription initiation sites, and alternative splicing produces several types of mRNA in steroidogenic organs. In this study, we show that four of five putative promoters are active in chicken ovary-derived cells. One of the active promoters, *LRH-1D*, was drastically repressed by WT1 (-KTS) and FOXL2. Moreover, LRH-1 and SF-1 weakly suppressed the activity. The expression of LRH-1 may be controlled by these factors in the chicken ovary.

Key words: LRH-1, SF-1, WT1, FOXL2, chicken

1. Introduction

Liver receptor homolog-1 (LRH-1) belongs to the orphan nuclear receptor family. In mammals, LRH-1[1)] has been identified independently by several groups, each of which referred to it differently: pancreatic homolog receptor 1[2)], fetoprotein transcription factor[3)], human B1-binding factor[4)], and *CYP7A* promoter binding factor[5)]. LRH-1 is expressed in organs derived from the endoderm, including the intestine, liver and exocrine pancreas and plays a predominant role in the development, bile-acid homeostasis, and reverse cholesterol transport. LRH-1 is also expressed in the steroidogenic organs[6,7)]. In mammals, LRH-1 may regulate the expression of aromatase in some steroidogenic organs such as the ovary[8)] and testis[9)], and stimulates progesterone biosynthesis by regulating the expression of 3b-hydroxysteroid dehydrogenase type II in the corpus luteum[10,11)]. These findings suggest that LRH-1 has an important role in mammalian female sexual functions.

Steroidogenic factor-1 (SF-1) is a paralog of LRH-1. In mammals, SF-1 has been identified as a master regulator that controls steroidogenic P-450 genes[12-14)] and plays a pivotal role in the development of steroidogenic organs: the adrenal glands and gonads[15,16)]. SF-1 is thought to play a key role in the development and physiology of steroidogenic organs. Recent research has revealed that LRH-1 shares roles in steroidogenesis with SF-1[6-11)].

In Aves, we identified cDNA clones for chicken LRH-1 and SF-1 as OR2.0 and OR2.1, respectively[17)]. Northern blotting showed that LRH-1 was expressed in the liver and adrenal glands at detectable levels. The RT-PCR profile

of the Zn-finger domain amplified with degenerated primers showed that LRH-1 was expressed in the adrenal glands at the same frequency as SF-1, and in the testis at a higher frequency than SF-1, but was not amplified from the ovary[17]. As the RT-PCR with degenerated primers could amplify various transcripts having the Zn-finger domain, the LRH-1 product must have occupied a negligibly small fraction of the entire products in the ovary. Furthermore, we clarified the genomic structure of the chicken SF-1 gene[18] and its promoter activity in chicken ovary-derived (COV1) cells. Recently, we showed the expression profiles of LRH-1 quantitatively, determined several transcription initiation sites of *LRH-1*, and showed the expression patterns of *LRH-1* splicing variants in the chicken steroidogenic organs[19]. Five putative promoter regions of chicken *LRH-1* included several transcription factor-recognition sequences, but it was not clear whether they functioned as promoters in steroidogenic organs.

If LRH-1 plays a vital role, as well as SF-1, in avian female sexual functions, it is important to clarify its role in the gonads, especially the ovary, to explain the sex determination mechanisms of birds. In this study, we describe the activities of putative chicken *LRH-1* promoters and the effects of several transcription factors in COV1 cells.

2. Materials and Methods

2.1. Construction of plasmid vectors

All promoter sequences were amplified by PCR from chicken genomic DNA (Table 1) and inserted into the plasmid vector pGL3-Basic (Promega). The *LRH-1D* and *SF-1* promoter (GenBank accession nos. **AB218279** and **AB018707**) was also inserted into the plasmid vector pEGFP-C1 (Clontech) instead of the CMV promoter. All open reading frames of transcription factors, *WT1* (GenBank accession nos. **AB033633** and **AB033634**), *LRH-1* (GenBank accession no. **AB002403**), and *SF-1* (GenBank accession no. **AB002404**), except for *FOXL2* were amplified by PCR from chicken embryonic or ovary cDNAs and inserted into the plasmid vector pTriEx-4 (Novagen) harboring the CAG promoter. The open reading frame of *FOXL2* (GenBank accession no. **AY487156**) was amplified by PCR from chicken genomic DNA. The sequences were verified by dideoxy sequencing using a DYEnamic ET Terminator Cycle Sequencing Kit (GE Healthcare Bio-sciences) and an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems).

Table 1. Sequence of primers for *LRH-1* promoters

Name (length)	Sequence
Promoter1aF (25)	CAGCTGGATTTTCCTCCAGTCACAC
Promoter1aR (26)	TGTTTACTTCGCTGGTGGGGTCCGTC
Promoter1bF (24)	GGCGCCCGAAGGAGAGGAGCCGCC
Promoter1bR (26)	CGCGCGGAAGCTCGCCTTATGTATTC
Promoter1cF (25)	GGGCCCTTCTTCTCCCCCGTCCGCC
Promoter1cR (26)	TTTAAACTGAGACACTGGGAAGAGA
Promoter1dF (23)	GTTCTGGGGCAGAGAGGCGGCAG
Promoter1dR (24)	GACAAGGCGGCTGCGGGCCGGGCT
Promoter1eF (23)	GGGCCCACAGCCCCTACCCACCT
Promoter1eR (30)	TATTGAGTGTAAATATACAGTACCATTGGG
Promoter1aF+ (30)	CCCTCGAGTAACAGTTTTCTGTTTTGATGA
Promoter1aR+ (39)	GCCAGACTGGAGAGCACTAGTTAGAATACC
Promoter1bF+ (30)	GGGGTACCGTGGGTATCTGCACCCAGGCGG
Promoter1bR+ (30)	CCGAGACCGTGCGGCCGCCGGGGAGCATCT
Promoter1cF+ (30)	GGGGTACCGTAAGGGGGGAACTGCACGGCG
Promoter1cR+ (30)	GGGGAGAAGAAGGGCCCGGCCCTCCACTCC

2.2. Cell culture and transfections

COV1 cells were prepared as described previously[18]. Briefly, the ovary was removed from a hen, from which small white follicles (SWF) and small yellow follicles (SYF) were collected. These tissues were minced with scissors and treated with collagenase (Sigma) at 4ºC overnight. The resulting cell suspensions were plated on tissue culture dishes and non-adhesive cells were removed after overnight incubation. Fibroblast-like cells thus obtained

were grown and maintained in Dulbecco's modified Eagle's medium (D-MEM, Sigma), supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37ºC in an atmosphere of 5% CO_2 in air. All transfections of COV1 cells were carried out in 24-well or 6-well plates coated with collagen type I using TransIT-LT1 reagent (Mirus Bio) according to the manufacturer's instructions.

2.3. Measurements of luciferase activity

Luciferase assays were performed using the Dual-Luciferase Assay System (Promega). In all cases, 2 x 10^4 COV1 cells were plated in a 24-well plate and, after 24 h, were transfected with 125 ng of internal control vector pRL-TK (Promega) and 375 ng of luciferase reporter vector, or 187.5 ng of luciferase reporter vector and 187.5 ng of transcription factor expression vector. The cells were lysed with 1 x Reporter Lysis Buffer (Promega) 48 h after transfection and subjected to an assay of luciferase activity with a Sirius Luminometer (Berthold). Firefly luciferase (Luc) activity was adjusted with reference to the activity of *Renilla* luciferase (Rluc) to normalize the efficiency of transfections.

2.4. Microscopy of fluorescence proteins

Fluorescence proteins were observed using an inverted fluorescence microscope IX71 (Olympus). In all cases, 6 x 10^4 COV1 cells were plated in a 6-well plate and, after 24 h, were transfected with 500 ng of control vector pDsRed2-C1 (Clontech) and 500 ng of EGFP reporter vector. The cells were observed under the fluorescence microscopy 48 h after transfection. All images were captured using a digital camera DP70 (Olympus).

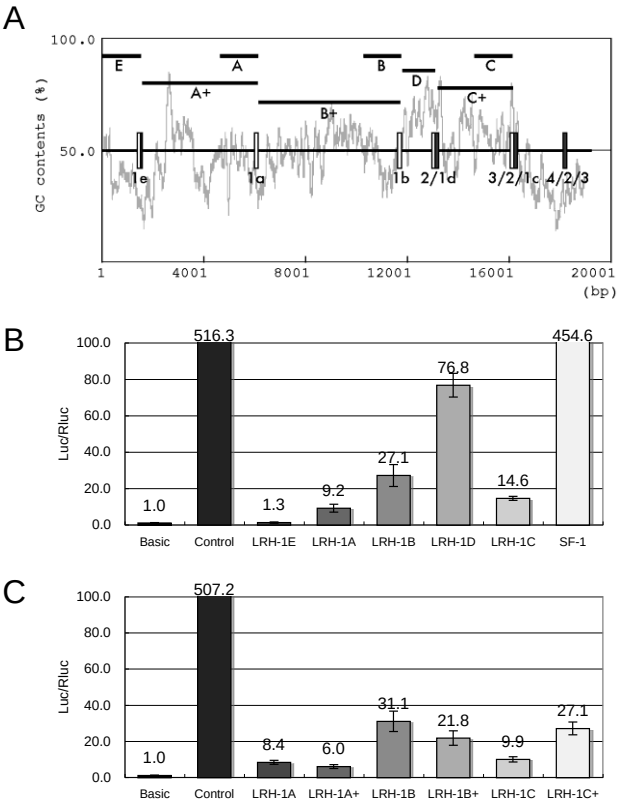

Fig. 1. Promoter activities of the chicken *LRH-1* gene in chicken ovary-derived (COV1) cells. (**A)** Structural organization of chicken *LRH-1* promoters (GenBank accession no. **AB218279**). The closed boxes indicate the locations and sizes of exons, including open reading frames. The opened boxes indicate the locations and sizes of exons, including 5' untranslated regions. Promoter regions used for promoter analyses are indicated by thick lines. GC contents were calculated and plotted by GENETYX-Mac software (GENETYX Inc.). (**B)** Activities of five putative promoters including 1kb upstream of exon promoters. (**C**) Activities of three promoters with or without expansion. Basic and Control indicate pGL3-Basic and pGL3-Control vector, respectively. The plasmid pGL3-Basic is promoter-less and pGL3-Control includes the SV40 promoter and enhancer. Activities from transfections with pGL3-Basic are taken as 1.0. The results shown are the mean (±SD) of at least three independent experiments performed in triplicate.

3. Results

3.1. Promoter activities of chicken LRH-1 gene in chicken ovary-derived cells

To determine the activities of multiple promoters in steroidogenic organs, transient expression assays were conducted in COV1 cells. These cells were mainly derived from theca cells, which are major steroidogenic cells in the ovary, and express several steroidogenic genes. All five promoter regions neighboring first exons, which

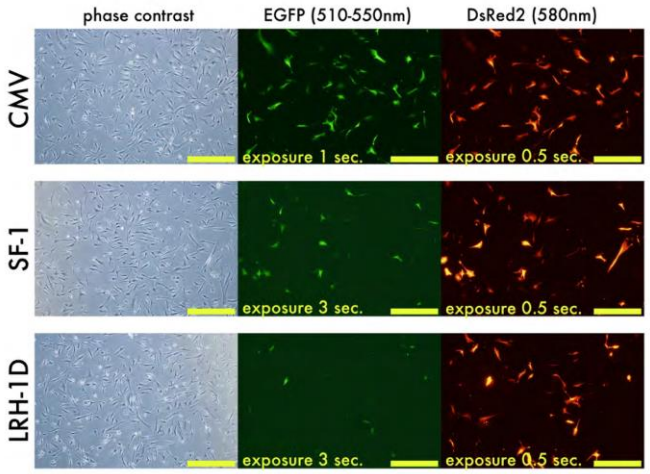

Fig. 2. Population of LRH-1- and SF-1-expressing cells in chicken ovary-derived (COV1) cells. The *EGFP* gene driven by CMV, *SF-1*, or *LRH-1D* promoter was introduced into COV1 cells. As an internal control for transfections, the DsRed-2 gene driven by the CMV promoter was co-transfected. Yellow bars indicate 500 µm.

we previously identified[19], were amplified by PCR (Fig. 1A). These regions include several GC-rich sequences. Exons 1d and 1b were separated by only 800 bp, so the region from the first initiation codon of exon 1d to the splicing donor site of exon 1b was used as the *LRH-1D* promoter. Upstream regions about 1 kb from the first initiation codon in exon 1e or 1c were used as the *LRH-1E* or *LRH-1C* promoter, and the upstream regions about 1 kb from 10 bp upstream of the splicing donor sites of exon 1a or 1b were used as the *LRH-1A* or *LRH-1B* promoter. The promoter of *SF-1* was the region about 1 kb from the splicing donor site of the first exon.

The *SF-1* promoter showed high activity equal to the SV40 promoter, pGL3-Control, in COV1 cells (Fig. 1B). On the other hand, *LRH-1* promoters indicated relatively low activities. Among five promoters, the *LRH-1D* promoter was the highest, but the activity was 17% that of the *SF-1* promoter. The activity of the *LRH-1B* promoter followed *LRH-1D*, and *LRH-1C* and *LRH-1A* showed extremely low activities. The activity of the *LRH-1E* promoter was equal to the promoter-less vector, pGL3-Basic.

To evaluate a wide promoter region, expanded regions of *LRH-1A*, *LRH-1B*, and *LRH-1C* promoters were used (Fig. 1A). The *LRH-1A* promoter extended to the splicing donor site of exon 1e (*LRH-1A*+). *LRH-1B*+ and *LRH-1C*+ also covered the splicing donor site of exon 1a or 1d, respectively. *LRH-1C*+ showed higher activity than *LRH-1C*, but the other promoters, *LRH-1A*+ and *LRH-1B*+, indicated slightly lower activity than *LRH-1A* or *LRH-1B*, respectively (Fig. 1C).

3.2. Population of LRH-1- or SF-1-expressing cells in chicken ovary-derived cells

COV1 cells are not an established cell line, so the cells were heterogeneous. To confirm the population of LRH-1- or SF-1-expressing cells, the green fluorescence protein (*GFP*) gene driven by *LRH-1D* or *SF-1* promoter was introduced into COV1 cells (Fig. 2). As an internal control, the red fluorescence protein (*RFP*) gene driven by the CMV promoter, pDsRed2-C1, was also introduced. GFP fluorescence driven by the *LRH-1D* or *SF-1* promoter was weaker than that driven by the CMV promoter, pEGFP-C1. GFP fluorescence driven by the *LRH-1D* promoter was weaker than that driven by the *SF-1* promoter because the *LRH-1D* promoter activities was low, although the population of LRH-1-expressing cells did not significantly differ from that of SF-1-expressing cells. The *GFP* gene driven by the *SF-1* promoter and the *RFP* gene driven by the *LRH-1D* promoter were co-transfected into COV1 cells and vice versa, the fluorescence of GFP and RFP was detected in the same cells (data not shown).

3.3. Co-expression of several transcription factor genes

Several transcription factors are known to be expressed in the chicken ovary. In COV1 cells, we detected the expression of Wilms' tumor suppression gene 1 (WT1), forkhead transcription factor L2 (FOXL2), SF-1, and LRH-1 by real-time RT-PCR (data not shown), and the chicken *LRH-1D* promoter included several transcription factor-recognition sites (Fig. 3A). To evaluate the effects of these transcription factors on the expression of LRH-1, the expression vectors expressing these transcription factors were co-transfected with pGL3-Basic harboring the *LRH-1D* promoter into COV1 cells (Fig. 3B). Open reading frames of chicken *WT1 (-KTS)*, *WT1 (+KTS)*, *FOXL2*, *LRH-1*, or *SF-1* were introduced into the expression vector, pTriEx-4, including a strong CAG promoter. WT1 (-KTS) and WT1 (+KTS) are major alternative splicing variants of WT1. WT1 (-KTS) and FOXL2 strongly repressed *LRH-1D* promoter activity, and LRH-1 also repressed it moderately. The repressive activities of WT1 (+KTS) and SF-1 were not strong, and the same effects were observed in chicken embryonic fibroblasts (CEF, data not shown).

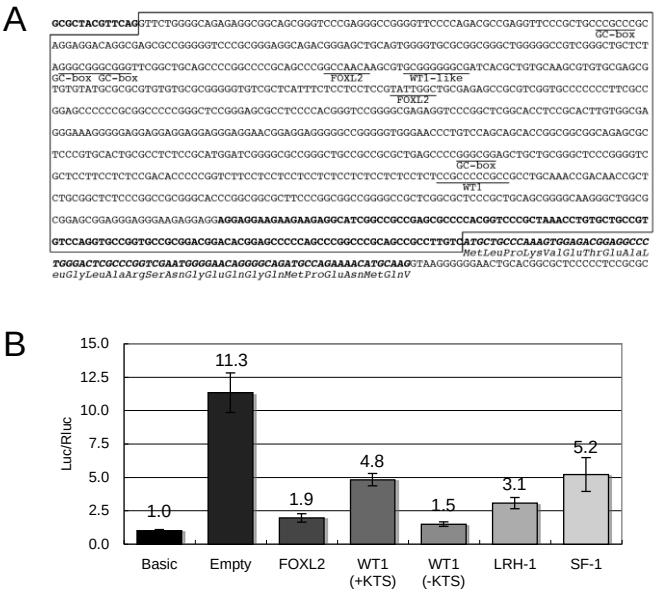

Fig. 3. Effects of several transcription factors on the activity of the chicken *LRH-1D* promoter. (**A**) A sequence of the genomic region including the chicken *LRH-1D* promoter. The sequence used for the promoter assay is boxed. Exons are shown in bold. (**B**) Activities of the *LRH-1D* promoter at co-transfection with several transcription factors. Basic indicates pGL3-Basic vector, and the plasmid pGL3-Basic is promoter-less. Empty indicates the co-transfection with the plasmid pTriEx-4 without insert. Activities from transfections with pGL3-Basic are taken as 1.0. The results shown are the mean (±SD) of at least three independent experiments in triplicate.

4. Discussion

We showed that four promoters of the chicken *LRH-1* gene could function in COV1 cells. The activity of the *LRH-1D* promoter was highest among *LRH-1* promoters; however, the splicing variant, transcribed from exon 1d, was a relatively major transcript in the adrenal glands, but not in the ovary and testis. In fish[20] and frogs[21], the corresponding exon is used as the first exon, and the promoter region of this exon could induce tissue-specific expression in transgenic frogs[22]. This promoter seems to be used widely among vertebrates, but its usage as the first exon has not been reported in mammals. Alternatively, the promoter activities of *LRH-1A*, *LRH-1B*, and *LRH-1C* were lower than that of *LRH-1D*. The splicing variants transcribed from exon 1a, 1b, and 1c, were major transcripts in the chicken ovary. COV1 cells may not reflect the expression profile in the ovary completely, or these promoters could function synergistically in steroidogenic organs. Recently, the splicing variant was identified by 5'-RACE in mouse embryonic stem cells R1 and was detected in several organs including the ovary and testis[23]. The transcript included a new alternative exon 1, but there was no homology between mammals and other vertebrates.

The chicken *LRH-1E* promoter is equivalent

to the mammalian *LRH-1* promoter in the liver and pancreas. In mammals, several transcription factors controlling the expression of LRH-1 have been identified[24-26], and important recognition sites, GATA, Nkx, NHF-1, NHF-4, DR4, and Pdx, are conserved in the chicken *LRH-1E* promoter (cf. GenBank accession no. **AB218279**). The *LRH-1E* promoter used in this study included the region necessary for the full activity of mammalian *LRH-1* promoter, but it did not function at all in COV1 cells. Since the splicing variant, transcribed from exon 1e, was not abundantly expressed in chicken gonads, the *LRH-1E* promoter might function specifically in other organs: the liver and pancreas.

The expression of SF-1 was higher than that of LRH-1 in the chicken ovary[17]. The promoter activity of *SF-1* was also higher than that of *LRH-1* in COV1 cells. In this study, we showed that the population of LRH-1-expressing cells did not differ from that of SF-1-expressing cells in COV1 cells, and the same cells could express both LRH-1 and SF-1. So these transcription factors functioned in the same cells. The expression balance of LRH-1 and SF-1 may control the expression of target genes in steroidogenic organs.

The *LRH-1D* promoter showed very GC-rich sequences and did not include typical TATA-box sequences (Fig. 3A). Moreover, *LRH-1D* promoter included WT1 and FOXL2 recognition sequences and GC boxes, so we examined the influence of several transcription factors on the *LRH-1D* promoter. WT1 is a zinc-finger protein expressed in the kidney and gonads[27]. WT1 (-KTS), the splicing variant of WT1, binds GC-rich sequences and represses the transcription of target genes[28]. WT1 (+KTS) is the splicing variant of WT1, which lacks DNA-binding activity. WT1 (-KTS) indicated drastic suppression of the *LRH-1D* promoter and WT1 (+KTS) showed weak suppression. WT1 (-KTS) may directly bind the *LRH-1D* promoter and suppress it. FOXL2 is a forkhead protein known as a transcriptional repressor[29]. The expression of FOXL2 correlates with that of aromatase in the chicken ovary[30]. FOXL2 also suppresses the *LRH-1D* promoter drastically and this effect may be caused by direct interaction. LRH-1 itself suppressed the *LRH-1D* promoter, but SF-1 suppressed it more weakly than LRH-1 despite lacking typical recognition sites. The *LRH-1D* promoter may therefore be controlled indirectly by negative feedback and SF-1 could also influence the expression of LRH-1. The low expression of LRH-1 in the chicken ovary might be caused by the high expression of SF-1.

In the chicken ovary, the *LRH-1* gene was mainly transcribed from four promoters. The strongest promoter was suppressed by WT1 (-KTS) and FOXL2 drastically, and by LRH-1 and SF-1 weakly. To clarify the mechanism and the physiological functions of this phenomenon, further study is required.

References

1) Tugwood, J.D., Issemann, I. and Green, S. (1991) LRH-1: A nuclear hormone receptor active in the absence of exogenous ligands. Unpublished and submitted to the GenBank as accession number **M81385**.
2) Becker-Andre, M., Andre, E. and DeLamarter, J.F. (1993) Identification of nuclear receptor mRNAs by RT-PCR amplification of conserved zinc-finger motif sequences. *Biochem. Biophys. Res. Commun*. 194, 1371-1379.
3) Galarneau, L., Pare, J.F., Allard, D., Hamel, D., Levesque, L., Tugwood, J.D., Green, S. and Belanger, L. (1996) The α_1-fetoprotein

locus is activated by a nuclear receptor of the *Drosophila* FTZ-F1 family. *Mol. Cell. Biol*. 16, 3853-3865.

4) Li, M., Xie, Y.H., Kong, Y.Y., Wu, X., Zhu, L. and Wang, Y. (1998) Cloning and characterization of a novel human hepatocyte transcription factor, hB1F, which binds and activates enhancer II of hepatitis B virus. *J. Biol. Chem*. 273, 29022-29031.
5) Nitta, M., Ku, S., Brown, C., Okamoto, A.Y. and Shan, B. (1999) CPF: an orphan nuclear receptor that regulates liver-specific expression of the human cholesterol 7α-hydroxylase gene. *Proc. Natl. Acad. Sci. USA* 96, 6660-6665.
6) Boerboom, D., Pilon, N., Behdjani, R., Silversides, D.W. and Sirois, J. (2000) Expression and regulation of transcripts encoding two members of the NR5A nuclear receptor subfamily of orphan nuclear receptors, steroidogenic factor-1 and NR5A2, in equine ovarian cells during the ovulatory process. *Endocrinology*. 141, 4647-4656.
7) Falender, A.E., Lanz, R., Malenfant, D., Belange, L. and Richards, J.S. (2003) Differential expression of steroidogenic factor-1 and FTF/LRH-1 in the rodent ovary. *Endocrinology*. 144, 3598-3610.
8) Liu, D.L., Liu, W.Z., Li, Q.L., Wang, H.M., Qian, D., Treuter, E. and Zhu, C. (2003) Expression and functional analysis of liver receptor homologue 1 as a potential steroidogenic factor in rat ovary. *Biol. Reprod*. 69, 508-517.
9) Pezzi, V., Sirianni, R., Chimento, A., Maggiolini, M., Bourguiba, S., Delalande, C., Carreau, S., Ando, S., Simpson, E.R. and Clyne, C.D. (2004) Differential expression of steroidogenic factor-1/adrenal 4 binding protein and liver receptor homolog-1 (LRH-1)/fetoprotein transcription factor in the rat testis: LRH-1 as a potential regulator of testicular aromatase expression. *Endocrinology*. 145, 2186-2196.
10) Peng, N., Kim, J.W., Rainey, W.E., Carr, B.R. and Attia, G.R. (2003) The role of the orphan nuclear receptor, liver receptor homologue-1, in the regulation of human corpus luteum 3β-hydroxysteroid dehydrogenase type II. *J. Clin. Endocrinol. Metab*. 88, 6020-6028.
11) Saxena D., Safi R., Little-Ihrig L. and Zeleznik A.J. (2004) Liver receptor homolog-1 stimulates the progesterone biosynthetic pathway during follicle-stimulating hormone-induced granulosa cell differentiation. *Endocrinology.* 145, 3821-3829.
12) Lala, D.S., Rice, D.A. and Parker, K.L. (1992) Steroidogenic factor 1, a key regulator of steroidogenic enzyme expression, is the mouse homolog of *fushi tarazu*-factor 1. *Mol. Endocrinol*. 6, 1249-1258.
13) Honda, S., Morohashi, K., Nomura, M., Takeya, H., Kitajima, M. and Omura, T. (1993) Ad4BP regulating steroidogenic P-450 gene is a member of steroid hormone receptor superfamily. *J. Biol. Chem*. 268, 7494-7502.
14) Lynch, J.P., Lala, D.S., Peluso, J.J., Luo, W., Parker, K.L. and White, B.A. (1993) Steroidogenic factor 1, an orphan nuclear receptor, regulates the expression of the rat aromatase gene in gonadal tissues. *Mol. Endocrinol*. 7, 776-786.
15) Luo, X., Ikeda, Y. and Parker, K.L. (1994) A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* 77, 481-490.
16) Sadovsky, Y., Crawford, P.A., Woodson, K.G., Polish, J.A. Clements, M.A. Tourtellotte, L.M. Simburger, K. and Milbrandt, J. (1995) Mice deficient in the orphan receptor steroidogenic factor 1 lack adrenal glands and gonads but express P450 side-chain-cleavage enzyme in the placenta and have normal embryonic serum levels of corticosteroids. *Proc. Natl. Acad. Sci. USA* 92,10939-10943.
17) Kudo, T. and Sutou, S. **(**1997) Molecular cloning of chicken FTZ-F1-related orphan receptors. *Gene*. 197, 261-268.
18) Kudo, T. and Sutou, S. (1999) Structural characterization of chicken SF-1/Ad4BP gene. *Gene*. 231, 33-40.

19) Kudo, T. and Sutou, S. (2006) Chicken LRH-1 gene is transcribed from multiple promoters in steroidogenic organs. *Gene*. 367, 38-45.

20) Lin, W, Wang, H.W., Sum, C., Liu, D., Hew, C.L. and Chung, B. (2000) Zebrafish *ftz-f1* gene has two promoters, is alternatively spliced, and is expressed in digestive organs. *Biochem J*. 348, 439-446.

21) Nakajima, T., Takase, M., Miura, I. and Nakamura, M. (2000) Two isoforms of FTZ-F1 messenger RNA: molecular cloning and their expression in the frog testis. *Gene*. 248, 203-212.

22) Asahi, H., Takase, M., Yuge, M., Matsui, K., Mori, M., Fujita, T. and Nakamura, M. (2002) Expression of FTZ-F1a in transgenic Xenopus embryos and oocytes. *Dev. Growth Differ*. 44, 509-516.

23) Gao, D.M., Wang, L.F., Liu, J., Kong, Y.Y., Wang, Y. and Xie, Y.H. (2006) Expression of mouse liver receptor homologue 1 in embryonic stem cells is directed by a novel promoter. *FEBS Lett*. 580, 1702-1708.

24) Paré, J.F., Roy, S., Galarneau, L. and Belanger, L. (2001) The mouse fetoprotein transcription factor (FTF) gene promoter is regulated by three GATA elements with tandem E box and Nkx motifs, and FTF in turn activates the *Hnf3β*, *Hnf4α*, and *Hnf1α* gene promoters. *J. Biol. Chem*. 276, 13136-13144.

25) Paré, J.F., Malenfant, D., Courtemanche, C., Jacob-Wagner, M., Roy, S., Allard, D. and Belanger, L. (2004) The fetoprotein transcription factor (*FTF*) gene is essential to embryogenesis and cholesterol homeostasis and is regulated by a DR4 element. *J. Biol. Chem*. 279, 21206-21216.

26) Annicotte, J.S., Fayard, E., Swift, G.H., Selander, L., Edlund, H., Tanaka, T., Kodama, T., Schoonjans, K. and Auwerx, J. (2003) Pancreatic-duodenal homeobox 1 regulates expression of liver receptor homolog 1 during pancreas development. *Mol. Cell. Biol*. 23, 6713-6724.

27) Pritchard-Jones, K., Fleming, S., Davidson, D., Bickmore, W., Porteous, D., Gosden, C., Bard, J., Buckler, A., Pelletier, J., Housman, D., van Heyningen, V. and Hastie, N. (1990) The candidate Wilms' tumour gene is involved in genitourinary development. *Nature* 346, 194-197.

28) Rauscher, F.J.III. (1993) The WT1 Wilms tumor gene product: a developmentally regulated transcription factor in the kidney that functions as a tumor suppressor. *FASEB J*. 7, 896-903.

29) Pisarska, M.D., Bae, J., Klein, C, and Hsueh, A.J. (2004) Forkhead L2 is expressed in the ovary and represses the promoter activity of the steroidogenic acute regulatory gene. *Endocrinology* 145, 3424-3433.

30) Govoroun, M.S., Pannetier, M., Pailhoux, E., Cocquet, J., Brillard, J.P., Couty, I., Batellier, F. and Cotinot, C. (2004) Isolation of chicken homolog of the FOXL2 gene and comparison of its expression patterns with those of aromatase during ovarian development. *Dev. Dyn*. 231, 859-870.