REVIEW

Molecular mechanism of chicken ovalbumin upstream promoter-transcription factor (COUP-TF) actions

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Abstract. Chicken ovalbumin upstream promoter-transcription factors (COUP-TFs) are one of the most characterized orphan receptors of the steroid/thyroid hormone receptor superfamily. COUP-TFs play important roles in the regulation of organogenesis, neurogenesis, and cellular differentiation during embryonic development. COUP-TFs were generally considered to be repressors of transcription, however, there are growing evidences that COUP-TFs can function as transcription activators. Here we will review the molecular mechanism of COUP-TFs as repressors and activators. Also, we will review the known biological function of COUP-TFI during development and differentiation. (Keio J Med 52 (3): 174–181, September 2003)

Key words: COUP-TF, transcription repressors, transcription activators, neurogenesis, organogenesis

Introduction

The nuclear receptor superfamily comprises a large group of ligand-activated transcription factors, including receptors for steroids, retinoids, and thyroid hormones.[1] Also included are a large number of structurally and functionally related transcription regulatory proteins termed orphan receptors, for which specific ligands have not been defined. Chicken ovalbumin upstream promoter-transcription factor (COUP-TF) is one of the most extensively studied orphan receptors. Two genes called COUP-TFI (also termed EAR3) and COUP-TFII (also termed ARP-1) have been identified in mammals. These are closely related transcription factors that are expressed in many places and are involved in the regulation of several important biological processes, such as neurogenesis, organogenesis, cell fate determination, and metabolic homeostasis.[1–8] Both genes show an exceptional homology and overlapping expression patterns, suggesting that they may serve redundant functions. However, each factor possesses its own distinct expression profile during development.[8] This review will focus on the advances that have been made towards understanding the molecular mechanism of COUP-TF actions and biological function of COUP-TFI during development and differentiation.

Molecular Mechanism of COUP-TF Actions

Repressor

COUP-TFs homodimerize or heterodimerize with retinoid X receptor (RXR) and a few other nuclear receptors and bind to a variety of response elements that contain imperfect AGGTCA direct or inverted repeats with various spacings.[9,10] Although COUP-TF was originally characterized as a transcriptional activator of the chicken ovalbumin gene,[11] COUP-TFs are generally considered to be repressors of transcription for other nuclear hormone receptors such as retinoic acid receptor (RAR), thyroid hormone receptor (TR), vitamin D receptor (VDR), peroxisome proliferator-activated receptor (PPAR), and hepatocyte nuclear factor 4 (HNF4).[10,12,13] There are four mechanisms that account for the repressive effects of COUP-TFs (Fig. 1).

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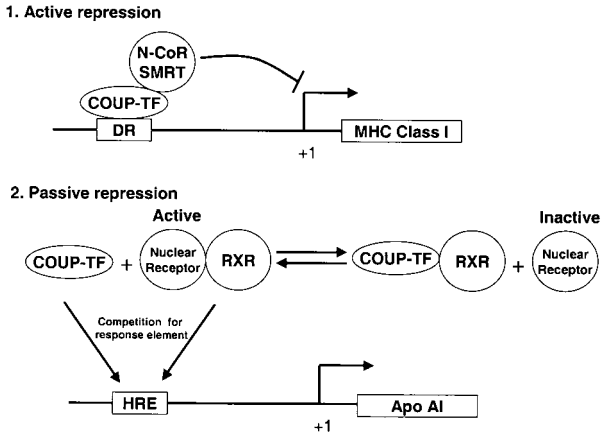

Fig. 1 Molecular mechanism of COUP-TFs as repressors. DR: direct repeats, HRE: hormone response element, MHC: major histocompatibility complex, ApoAI: apolipoprotein AI.

The competition for occupancy of the binding sites: COUP-TFs have been shown to bind to a variety of direct repeats, including DR1, DR3, DR4, and DR5 of the AGGTCA motif, which are response elements of PPAR, VDR, TR, and RAR, respectively.[14–16] It has been demonstrated that COUP-TFs repress the hormonal induction of target genes by PPAR, VDR, TR, and RAR in transient transfection assays through direct competition with VDR, TR, and RAR for the available binding sites.[14,15] The repression is released by increasing the expression of RAR, which suggests that COUP-TFs negatively regulate retinoid responses by competing for binding to the retinoid response elements of the target reporter. COUP-TFs have also been demonstrated to interfere with the transactivation of TR, RXR, and PPAR through a similar mechanism.[14–16] In addition, COUP-TFs have been shown to inhibit the transactivation of steroidogenic factor 1 (SF1) and HNF4 due to mutually exclusive binding to the promoter of many genes.[17–19] Finally, COUP-TF has been shown to antagonize ER activation of the lactoferrin and oxytocin promoters by binding to a binding site that overlaps with the estrogen response element.[17,20]

The competition for RXR: It is well known that RXR is a universal heterodimeric partner of RAR, TR, VDR, PPAR, and other orphan receptors.[6] Homodimers of RAR, TR, VDR, and PPAR either bind poorly or not at all to their cognate response elements. The heterodimeric receptors can bind to the cognate response elements with high affinity through association with RXR, thus, enhance the transactivation potential of this group of receptors. Because the direct repeat recognition sequence is asymmetric, it has been shown that RXR occupies the 5′ half-site while the other partner binds the downstream 3′ half-site, which confers the hormone responsiveness.[6] RXR can also bind to DR1 elements as a homodimer and as a heterodimer with RAR and PPAR. The RXR homodimer is an activator that responds to 9-*cis*-retinoic acid. RXR/PPAR heterodimers respond to both 9-*cis*-retinoic acid- and PPAR-specific ligands. However, RAR/RXR heterodimers, in which RAR binds to the 5′-half-site of DR1 element, are transactivationally inactive. It has been shown that RAR and TR bind to a corepressor [either silencing mediator for retinoid and thyroid hormone receptors (SMRT) or nuclear receptor corepressor (N-CoR)] in the absence of hormone.[21,22] Binding of these corepressors is necessary for receptors to silence the promoter activity. Upon binding hormone, the corepressor is released, and, thus, silencing activity of receptors is abolished. However, when RAR/RXR binds to DR1, the retinoic acid ligand is not able to release the corepressor from RAR; therefore, RAR/RXR heterodimer is not able to activate the DR1 reporter.[23]

Although COUP-TFs exist in solution as homodimers and fail to form stable heterodimers with RXR in coimmunoprecipitation assays,[10,24] they readily form DNA-binding heterodimers with RXR.[10,15,16] Therefore, COUP-TFs are able to sequester the common heterodimerization partner RXR and reduce the available concentrations of RXR.[10,14–16,25] The loss of RXR indirectly decreases the DNA-binding affinity of TR, VDR, RAR, and PPAR and thereby interferes with the potential of this subgroup of receptors to transactivate their target genes.[14,15,26] This notion is further verified by the relief of COUP-TF inhibition when RXR is overexpressed.[14] In addition, it has been demonstrated that COUP-TFs form heterodimers with TR and RAR and disrupt their functions.[13,27,28] Thus, the ability of COUP-TFs to form heterodimers with RXR, TR, and RAR may contribute significantly to the negative regulatory role of COUP-TFs in modulating hormone responsiveness of a large number of receptors of the TR and RAR subfamily.[6]

Active repression: Similar to unliganded nuclear hormone receptors, COUP-TFs have been shown to repress basal transcriptional activity of a number of thymidine kinase reporters containing DR3, DR4, or DR5 hormone response elements.[10,14] This silencing of basal transcriptional activity is response element specific and is unlikely due to squelching of TFIIB, which interacts with COUP-TFs or other general transcription factors, since reporter genes lacking COUP-TF binding sites show little COUP-TF-mediated repression.[13] Subsequently, it has been demonstrated that COUP-TFs,

similar to TR and RAR, possess an active silencing domain within the C terminus of the putative ligand binding domain (LBD). This repressor domain can be transferred to a heterologous GAL4 DNA binding domain (DBD) and can be shown to retain its ability to repress basal transcriptional activity.

In addition, Leng *et al*. have shown that COUP-TFs can function as an active repressor in a dose-dependent manner to inhibit transactivation mediated by acidic (Gal4-RII), glutamine-rich (Gal4-ftzQ), proline-rich (Gal4-CTF1P), and Ser/Thr-rich (Gal4-ZenST) transactivators.[13] The active repressor function of COUP-TFs is position independent, *i.e.* the binding sites of COUP-TFs can either be localized upstream of the activator binding site or downstream of the reporter gene without significantly affecting the active repression. The fact that COUP-TFs can repress such diverse groups of transactivators suggests that it is unlikely due to COUP-TFs directly quenching these transactivators or interfering with their interaction with their respective targets. Rather, it is likely that COUP-TFs interact with a common target, a putative corepressor(s) that mediates their repression. Shibata *et al*. demonstrated that corepressors are involved in COUP-TFI-mediated gene silencing, and that both N-CoR and SMRT can function as corepressors for COUP-TFI in mammalian cells.[29] Therefore, COUP-TFI can function as a repressor *in vivo* by utilizing corepressors that are common for members of the TR and RAR subfamily. In addition, it was discovered that in adenovirus type 12 transformed cells, COUP-TF associates with histone deacetylase through its C-terminal repression domain and that this association apparently plays an important role in the down-regulation of major histocompatibility complex (MHC) class I transcription.[30] Thus, COUP-TFs can repress transcription through a mechanism similar to that described for nuclear receptors RAR/RXR or TR/RXR, which associate with histone deacetylases in the absence of their specific ligands.[31]

Transrepression: COUP-TFs can also repress transcription by directly binding to the LBD of nuclear hormone receptors, a process called transrepression.[13,32] Leng *et al*. have demonstrated that COUP-TFI represses transcriptional activity induced by fusion proteins between the GAL4 DBD and LBD of TR, RXR, or RAR. Based on these results, they proposed a mechanism for transrepression by COUP-TFs invovling heterodimerization of COUP-TF proteins with other nuclear hormone receptors, such as TR, RAR, or RXR.[13] Therefore, COUP-TFs can be tethered to DNA in the absence of their cognate response elements *via* LBD-LBD interactions with other receptors such as TR, RAR, and RXR to transrepress the ligand-dependent transactivation of the nuclear receptors. Also, Achatz *et al*. reported that transrepression is the predominant mechanism underlying repressor activity of ARP-1/COUP-TFII, and this mechanism most likely involves interaction of protein with one or several transcriptional coactivator proteins which are employed by various liver-enriched transactivators such as HNF-4, HNF-3, and C/EBP but not by ubiquitous factors such as Sp1 or ATF.[32]

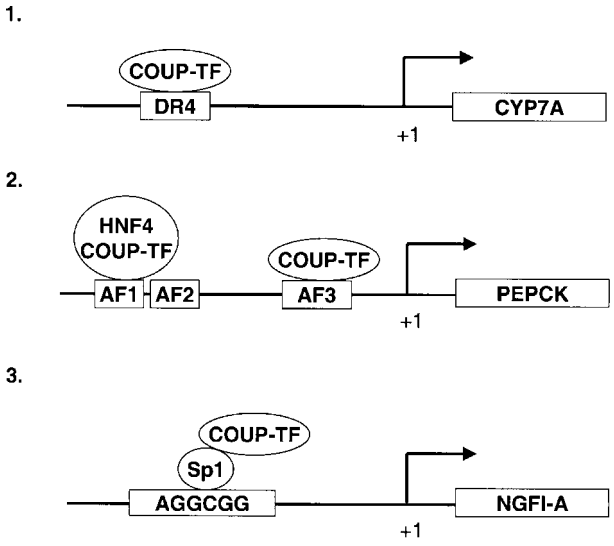

Fig. 2 Molecular mechanism of COUP-TFs as activators. 1: direct activation by binding to DNA response element, 2: indirect activation by acting as accessory factors for transcription activation, 3: activation through protein-protein interaction with a DNA-bound factor. DR: direct repeats, AF: accessory factor elements, CYP7A: cholesterol 7α-hydroxylase, PEPCK: phosphoenolpyruvate carboxykinase.

Activator

COUP-TFs can also function as positive regulators for many different genes. Positive regulation by COUP-TFs can be carried out by at least three different molecular mechanisms of activation of gene expression by COUP-TFs (Fig. 2). First, COUP-TF activates transcription by binding to a nuclear receptor DNA response element and directly activating gene expression. For example, COUP-TFII stimulates the transcriptional activity of the rat cholesterol 7α-hydroxylase (CYP7A) promoter by binding to the nucleotide sequence located between −74 and −54 (relative to the transcription start site), which contains a direct repeat of two hormone response element half-sites separated by 4 nucleotides (a DR4).[33] Similarly, in the arrestin gene promoter, a DR-7 element mediates the positive transcriptional effect of COUP-TF.[34]

Second, COUP-TF activates transcription by binding to a DNA element and indirectly influencing expression

in the context of several other transcription factors, as in the phosphoenolpyruvate carboxykinase (PEPCK) gene glucocorticoid response unit (GRU).[35,36]

Finally, COUP-TF activates transcription through protein-protein interaction with a DNA-bound factor, such as with HNF-4 in the HNF-1α gene promoter,[37] with Sp1 in the trout estrogen receptor,[38] the phosphoenolpyruvate carboxykinase,[35] the vHNF1,[39] the human immunodeficiency virus long terminal repeat,[40] and NGFI-A[41] genes.

Based on three different molecular mechanisms of gene activation by COUP-TFs, Sugiyama *et al*. have characterized the domain required for COUP-TF-mediated transcriptional activation of PEPCK gene and have determined that SRC-1 and GRIP1 bind to this domain and serve as coactivators.[42] Similarly, for the NGFI-A gene, COUP-TF enhances transcription by recruiting coactivator SRC-1 through its interaction with Sp1.[41]

Recently, Metivier *et al.* studied the molecular basis underlying the positive action of COUP-TFI on ERα activity and suggested a novel pathway in which the formation of a tight ERα-COUP-TFI intermediate complex through COUP-TFI DBD and ERLBD resulted in an increased recruitment of ERK2/p42^{MAPK}, phosphorylation of the hERα on Ser 118 and enhanced its transcriptional activity.[43]

COUP-TFs can act as repressors or activators of gene expression. However, the mechanisms underlying functional duality are unknown. Some of this functional duality might depend on the repertoire of coregulator proteins which interact with COUP-TF. Evidence suggests that the function of many nuclear hormone receptors is dependent upon, or modulated by, the actions of both common and distinct receptor binding cofactors that differentially recognize liganded and unliganded receptors.[21,22,44–49] Most of the auxiliary factors so far identified act as corepressors (e.g. N-CoR, SMRT, TRUP, and TRIP1). However, in a few cases, receptor-selective, positively acting coactivators (e.g. RIP140, SRC-1, and CBP/p300) have been identified. Marcus *et al*. have identified a factor that bound COUP-TFII *in vitro* and allowed COUP-TFII to act as a transcriptional activator in mammalian cells. This factor is a recently reported ligand of the tyrosine kinase signaling molecule p56^{lck}. These results, if proved to be correct, suggest that this factor mediates cross-talk between mitogenic and nuclear hormone receptor signal transduction pathways.[50] In addition, the bifunctional activity of COUP-TF may also depend on the promoter contexts of target genes. In this respect, a recent paper describes that corepressor SMRT functions as a coactivator for TR from a negative hormone response element.[51] The mechanism on how this works is not clear.

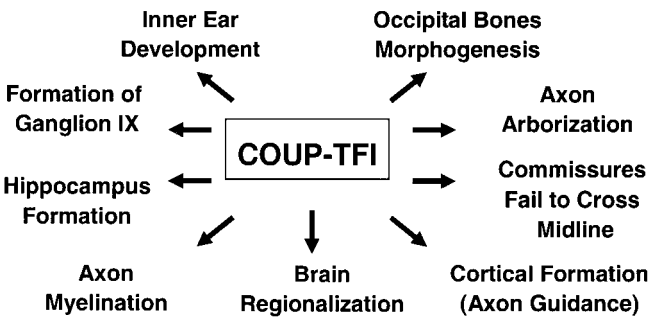

Fig. 3 Biological functions of COUP-TFI during development and differentiation.

Biological Function of COUP-TFI During Development and Differentiation

The molecular biology and expression studies have revealed several possible functions of COUP-TFs during development and differentiation. Here we will concentrate our review on COUP-TFI and describe several of its physiological functions (Fig. 3).

The role of COUP-TFI in neurogenesis

COUP-TFI and COUP-TFII[3] have been extensively studied, both in terms of biochemical properties and tissue distribution, with a particular emphasis on developmental processes.[9] In all the species that were examined for the presence of COUP-TF during development (from sea urchin to mouse), expression of this orphan receptor was clearly associated with neurogenesis.[34,52–56] Connor *et al*. demonstrated that COUP-TFI overexpression resulted in decreased contact stability between neurites and substrate cells and suggested COUP-TFI is involved in the regulation of cell-cell contacts.[57] Recent studies using gene targeting suggest that COUP-TFI is involved in modulation of axonal growth.[58,59] Indeed, targeted disruption of mouse COUP-TFI results in decreased arborization of spinal nerves,[58] in abnormal morphogenesis of the ninth cranial nerve and ganglion,[58] and in defects in the guidance of axons emanating from thalamic neurons that normally project to cortical layer IV.[59] Finally, disruption of COUP-TFI in mice results in the improper brain regionalization. These results strongly suggest that COUP-TFI is an important component in the regulation of neurogenesis and cellular differentiation during embryonic development in several organisms.

Several studies have pointed out the possibility that COUP-TF genes could be part of retinoid signaling pathways both *in vivo* and in cell culture systems.[60–63] Notably, up-regulation of COUP-TFI and COUP-TFII genes occurs during the differentiation programs induced by retinoic acid (RA) in mouse teratocarcinoma

cells such as P19 embryonal carcinoma (EC) cells.[61] Pluripotent P19 EC cells can be induced to differentiate into all three germ layer derivatives (*i.e.* ectoderm, endoderm, and mesoderm) when appropriate inducers and culture conditions are used.[64–66] When P19 cells are grown as aggregates, RA induces a neuroectodemal-like differentiation pathway.[64] Therefore, P19 cells have been widely used to screen for genes involved in neuronal differentiation.[67–69] Adam *et al*. studied the functional involvement of COUP-TFI in RA-induced neuronal differentiation of P19 cells through two approaches: 1) deregulated expression of COUP-TFI, and 2) inactivation of endogenous COUP-TF by means of a dominant negative COUP-TFI mutant. They reported that a too early (or too high) expression of wild-type COUP-TFI impedes neural differentiation and inhibition of endogenous COUP-TF by expression of a dominant-negative COUP-TFI mutant results in a strengthening of cell-cell contacts, decreased axonal growth, and slower migration of neurons. These data provide evidence that COUP-TFs are regulators of cell adhesion mechanisms required for the differentiation of embryonal carcinoma.[70]

The role of COUP-TFI in neocortex regionalization

Regionalization of the cerebral cortex can be divided into at least two steps: (1) an early regionalization phase in which cortical neurons establish their regional identity through regulating gene expression in a cell-autonomous manner and (2) a refinement phase in which extrinsic influences from subcortical areas, including thalamocortical inputs, shape and maintain the cortical subdivisions. Recent studies showed that neocortical regional identity was shifted in Pax6 and Emx2 mutant mice, indicating that Pax6 and Emx2 are two such intrinsic factors that regulate the regional expression of marker genes and specify cortical identity.[71,72] Zhou *et al*. studied the role of COUP-TFI in early neocortical regionalization and demonstrated that COUP-TFI is required for the region-specific expression of many marker genes, as well as the precise axonal projections between the thalamus and the cortex. These data indicate that COUP-TFI is a regulatory factor that works in concert with Pax6 and Emx2 to specify cortical identity.[73]

The role of COUP-TFI in organogenesis

Retinoids are known to regulate the expression of Hox genes, which play a major role in pattern formation and bone morphogenesis.[74–76] Because COUP-TFI is hypothesized to antagonize retinoid function and its expression is known to be regulated by retinoids, it is of interest to assess whether loss of COUP-TFI function in the null mutants will affect bone formation. Qiu *et al*. reported that the left or the right exoccipital bone is prematurely fused to the basioccipital bone in 98% of the COUP-TF1 null mutants.[58] This result suggests that COUP-TFI plays a major role in the development of these bones.

In addition, several other abnormalities including malformation of hippocampus, faulty axon myelination, and inner ear defects were also observed in the COUP-TFI null mutants.[58] These results strongly suggest that COUP-TFI is a crucial component in the regulation of cellular differentiation during embryonic development in several organs.

Future Directions

Further characterization of COUP-TFI activity *in vivo* and analysis of its target genes will allow a better understanding of its role in development and differentiation. Moreover, the role of COUP-TFI beyond the embryonic stage is very intriguing and remains to be elucidated. The discovery by Brian Sauer that the Cre recombinase of bacteriophage P1 could be used to rearrange the eukaryotic genome suggested a mechanism that would remove the restriction against introducing a mutation specifically into somatic cells.[77] The first report of the successful application of this approach, inactivating the DNA polymerase β gene in T cells, was published in 1994.[78] Two lines of mice are required for the Cre-loxP approach to work. In one line, the targeted allele is flanked by two loxP sequences, which target the flanked region for excision by the Cre recombinase. In another line, the expression of Cre is controlled by a tissue-specific promoter. Crossing the two lines of mice leads to an animal lacking the gene only in tissues that express Cre, while leaving the gene functional in all other tissues. The advantages of such a system are obvious: the function of genes necessary for fetal development can be preserved, allowing the function(s) of the gene to be examined during adulthood. The perinatal lethality of COUP-TFI null mutants limit the study of the function of COUP-TFI after birth. Therefore, the future generation of conditional knockouts using Cre-loxP system to delete COUP-TFI gene in a particular cell or tissue of interest will lead to a better understanding the role of COUP-TFI.

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References

1. Tsai MJ, O'Malley BW: Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 1994; 63: 451–486

2. Wang LH, Tsai SY, Cook RG, Beattie WG, Tsai MJ, O'Malley BW: COUP transcription factor is a member of the steroid receptor superfamily. Nature 1989; 340: 163–166
3. Ladias JA, Karathanasis SK: Regulation of the apolipoprotein AI gene by ARP-1, a novel member of the steroid receptor superfamily. Science 1991; 251: 561–565
4. Wang LH, Ing NH, Tsai SY, O'Malley BW, Tsai MJ: The COUP-TFs compose a family of functionally related transcription factors. Gene Expr 1991; 1: 207–216
5. Kastner P, Mark M, Chambon P: Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? Cell 1995; 83: 859–869
6. Mangelsdorf DJ, Evans RM: The RXR heterodimers and orphan receptors. Cell 1995; 83: 841–850
7. Ritchie HH, Wang LH, Tsai S, O'Malley BW, Tsai MJ: COUP-TF gene: a structure unique for the steroid/thyroid receptor superfamily. Nucleic Acids Res 1990; 18: 6857–6862
8. Qiu YH, Tsai SY, Tsai MJ: COUP-TF: An orphan member of the steroid/thyroid hormone receptor superfamily. Trends Endocrinol Metab 1994; 5: 234–239
9. Tsai SY, Tsai MJ: Chick ovalbumin upstream promoter-transcription factors (COUP-TFs): coming of age. Endocr Rev 1997; 18: 229–240
10. Cooney AJ, Tsai SY, O'Malley BW, Tsai MJ: Chicken ovalbumin upstream promoter transcription factor (COUP-TF) dimers bind to different GGTCA response elements, allowing COUP-TF to repress hormonal induction of the vitamin D3, thyroid hormone, and retinoic acid receptors. Mol Cell Biol 1992; 12: 4153–4163
11. Sagami I, Tsai SY, Wang H, Tsai MJ, O'Malley BW: Identification of two factors required for transcription of the ovalbumin gene. Mol Cell Biol 1986; 6: 4259–4267
12. Ladias JA, Hadzopoulou-Cladaras M, Kardassis D, Cardot P, Cheng J, Zannis V, Cladaras C: Transcriptional regulation of human apolipoprotein genes ApoB, ApoCIII, and ApoAII by members of the steroid hormone receptor superfamily HNF-4, ARP-1, EAR-2, and EAR-3. J Biol Chem 1992; 267: 15849–15860
13. Leng X, Cooney AJ, Tsai SY, Tsai MJ: Molecular mechanisms of COUP-TF-mediated transcriptional repression: evidence for transrepression and active repression. Mol Cell Biol 1996; 16: 2332–2340
14. Cooney AJ, Leng X, Tsai SY, O'Malley BW, Tsai MJ: Multiple mechanisms of chicken ovalbumin upstream promoter transcription factor-dependent repression of transactivation by the vitamin D, thyroid hormone, and retinoic acid receptors. J Biol Chem 1993; 268: 4152–4160
15. Kliewer SA, Umesono K, Heyman RA, Mangelsdorf DJ, Dyck JA, Evans RM: Retinoid X receptor-COUP-TF interactions modulate retinoic acid signaling. Proc Natl Acad Sci USA 1992; 89: 1448–1452
16. Tran P, Zhang XK, Salbert G, Hermann T, Lehmann JM, Pfahl M: COUP orphan receptors are negative regulators of retinoic acid response pathways. Mol Cell Biol 1992; 12: 4666–4676
17. Wehrenberg U, Ivell R, Jansen M, von Goedecke S, Walther N: Two orphan receptors binding to a common site are involved in the regulation of the oxytocin gene in the bovine ovary. Proc Natl Acad Sci USA 1994; 91: 1440–1444
18. Bakke M, Lund J: Mutually exclusive interactions of two nuclear orphan receptors determine activity of a cyclic adenosine 3′,5′-monophosphate-responsive sequence in the bovine CYP17 gene. Mol Endocrinol 1995; 9: 327–339
19. Schaeffer E, Guillou F, Part D, Zakin MM: A different combination of transcription factors modulates the expression of the human transferrin promoter in liver and Sertoli cells. J Biol Chem 1993; 268: 23399–23408
20. Liu Y, Yang N, Teng CT: COUP-TF acts as a competitive repressor for estrogen receptor-mediated activation of the mouse lactoferrin gene. Mol Cell Biol 1993; 13: 1836–1846
21. Chen JD, Evans RM: A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 1995; 377: 454–457
22. Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK, *et al*: Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 1995; 377: 397–404
23. Kurokawa R, DiRenzo J, Boehm M, Sugarman J, Gloss B, Rosenfeld MG, Heyman RA, Glass CK: Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding. Nature 1994; 371: 528–531
24. Wang LH, Tsai SY, Sagami I, Tsai MJ, O'Malley BW: Purification and characterization of chicken ovalbumin upstream promoter transcription factor from HeLa cells. J Biol Chem 1987; 262: 16080–16086
25. Widom RL, Rhee M, Karathanasis SK: Repression by ARP-1 sensitizes apolipoprotein AI gene responsiveness to RXR alpha and retinoic acid. Mol Cell Biol 1992; 12: 3380–3389
26. Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM: Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. Nature 1992; 358: 771–774
27. Berrodin TJ, Marks MS, Ozato K, Linney E, Lazar MA: Heterodimerization among thyroid hormone receptor, retinoic acid receptor, retinoid X receptor, chicken ovalbumin upstream promoter transcription factor, and an endogenous liver protein. Mol Endocrinol 1992; 6: 1468–1478
28. Casanova J, Helmer E, Selmi-Ruby S, Qi JS, Au-Fliegner M, Desai-Yajnik V, Koudinova N, Yarm F, Raaka BM, Samuels HH: Functional evidence for ligand-dependent dissociation of thyroid hormone and retinoic acid receptors from an inhibitory cellular factor. Mol Cell Biol 1994; 14: 5756–5765
29. Shibata H, Nawaz Z, Tsai SY, O'Malley BW, Tsai MJ: Gene silencing by chicken ovalbumin upstream promoter-transcription factor I (COUP-TFI) is mediated by transcriptional corepressors, nuclear receptor-corepressor (N-CoR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT). Mol Endocrinol 1997; 11: 714–724
30. Smirnov DA, Hou S, Ricciardi RP: Association of histone deacetylase with COUP-TF in tumorigenic Ad12-transformed cells and its potential role in shut-off of MHC class I transcription. Virology 2000; 268: 319–328
31. Perlmann T, Evans RM: Nuclear receptors in Sicily: all in the famiglia. Cell 1997; 90: 391–397
32. Achatz G, Holzl B, Speckmayer R, Hauser C, Sandhofer F, Paulweber B: Functional domains of the human orphan receptor ARP-1/COUP-TFII involved in active repression and transrepression. Mol Cell Biol 1997; 17: 4914–4932
33. Stroup D, Crestani M, Chiang JY: Orphan receptors chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) and retinoid X receptor (RXR) activate and bind the rat cholesterol 7alpha-hydroxylase gene (CYP7A). J Biol Chem 1997; 272: 9833–9839
34. Lu XP, Salbert G, Pfahl M: An evolutionary conserved COUP-TF binding element in a neural-specific gene and COUP-TF expression patterns support a major role for COUP-TF in neural development. Mol Endocrinol 1994; 8: 1774–1788
35. Hall RK, Sladek FM, Granner DK: The orphan receptors COUP-TF and HNF-4 serve as accessory factors required for induction of phosphoenolpyruvate carboxykinase gene transcription by glucocorticoids. Proc Natl Acad Sci USA 1995; 92: 412–416

36. Scott DK, Mitchell JA, Granner DK: The orphan receptor COUP-TF binds to a third glucocorticoid accessory factor element within the phosphoenolpyruvate carboxykinase gene promoter. J Biol Chem 1996; 271: 31909–31914
37. Ktistaki E, Talianidis I: Chicken ovalbumin upstream promoter transcription factors act as auxiliary cofactors for hepatocyte nuclear factor 4 and enhance hepatic gene expression. Mol Cell Biol 1997; 17: 2790–2797
38. Lazennec G, Kern L, Valotaire Y, Salbert G: The nuclear orphan receptors COUP-TF and ARP-1 positively regulate the trout estrogen receptor gene through enhancing autoregulation. Mol Cell Biol 1997; 17: 5053–5066
39. Lazennec G, Kern L, Valotaire Y, Salbert G: The nuclear orphan receptors COUP-TF and ARP-1 positively regulate the trout estrogen receptor gene through enhancing autoregulation. Mol Cell Biol 1997; 17: 5053–5066
40. Rohr O, Aunis D, Schaeffer E: COUP-TF and Sp1 interact and cooperate in the transcriptional activation of the human immunodeficiency virus type 1 long terminal repeat in human microglial cells. J Biol Chem 1997; 272: 31149–31155
41. Pipaon C, Tsai SY, Tsai MJ: COUP-TF upregulates NGFI-A gene expression through an Sp1 binding site. Mol Cell Biol 1999; 19: 2734–2745
42. Sugiyama T, Wang JC, Scott DK, Granner DK: Transcription activation by the orphan nuclear receptor, chicken ovalbumin upstream promoter-transcription factor I (COUP-TFI). Definition of the domain involved in the glucocorticoid response of the phosphoenolpyruvate carboxykinase gene. J Biol Chem 2000; 275: 3446–3454
43. Metivier R, Gay FA, Hubner MR, Flouriot G, Salbert G, Gannon F, Kah O, Pakdel F: Formation of an hER alpha-COUP-TFI complex enhances hER alpha AF-1 through Ser118 phosphorylation by MAPK. EMBO J 2002; 21: 3443–3453
44. Onate SA, Tsai SY, Tsai MJ, O'Malley BW: Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 1995; 270: 1354–1357
45. Cavailles V, Dauvois S, L'Horset F, Lopez G, Hoare S, Kushner PJ, Parker MG: Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. EMBO J 1995; 14: 3741–3751
46. Le Douarin B, Zechel C, Garnier JM, Lutz Y, Tora L, Pierrat P, Heery D, Gronemeyer H, Chambon P, Losson R: The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-raf in the oncogenic protein T18. EMBO J 1995; 14: 2020–2033
47. Lee JW, Ryan F, Swaffield JC, Johnston SA, Moore DD: Interaction of thyroid-hormone receptor with a conserved transcriptional mediator. Nature 1995; 374: 91–94
48. Kurokawa R, Soderstrom M, Horlein A, Halachmi S, Brown M, Rosenfeld MG, Glass CK: Polarity-specific activities of retinoic acid receptors determined by a co-repressor. Nature 1995; 377: 451–454
49. Burris TP, Nawaz Z, Tsai MJ, O'Malley BW: A nuclear hormone receptor-associated protein that inhibits transactivation by the thyroid hormone and retinoic acid receptors. Proc Natl Acad Sci USA 1995; 92: 9525–9529
50. Marcus SL, Winrow CJ, Capone JP, Rachubinski RA: A $p56^{lck}$ ligand serves as a coactivator of an orphan nuclear hormone receptor. J Biol Chem 1996; 271: 27197–27200
51. Berghagen H, Ragnhildstveit E, Krogsrud K, Thuestad G, Apriletti J, Saatcioglu F: Corepressor SMRT functions as a coactivator for thyroid hormone receptor T3Ralpha from a negative hormone response element. J Biol Chem 2002; 277: 49517–49522
52. Fjose A, Nornes S, Weber U, Mlodzik M: Functional conservation of vertebrate seven-up related genes in neurogenesis and eye development. EMBO J 1993; 12: 1403–1414
53. Lutz B, Kuratani S, Cooney AJ, Wawersik S, Tsai SY, Eichele G, Tsai MJ: Developmental regulation of the orphan receptor COUP-TF II gene in spinal motor neurons. Development 1994; 120: 25–36
54. Pereira FA, Qiu Y, Tsai MJ, Tsai SY: Chicken ovalbumin upstream promoter transcription factor (COUP-TF): expression during mouse embryogenesis. J Steroid Biochem Mol Biol 1995; 53: 503–508
55. Qiu Y, Cooney AJ, Kuratani S, DeMayo FJ, Tsai SY, Tsai MJ: Spatiotemporal expression patterns of chicken ovalbumin upstream promoter-transcription factors in the developing mouse central nervous system: evidence for a role in segmental patterning of the diencephalon. Proc Natl Acad Sci USA 1994; 91: 4451–4455
56. Vlahou A, Gonzalez-Rimbau M, Flytzanis CN: Maternal mRNA encoding the orphan steroid receptor SpCOUP-TF is localized in sea urchin eggs. Development 1996; 122: 521–526
57. Connor H, Nornes H, Neuman T: Expression screening reveals an orphan receptor chick ovalbumin upstream promoter transcription factor I as a regulator of neurite/substrate-cell contacts and cell aggregation. J Biol Chem 1995; 270: 15066–15070
58. Qiu Y, Pereira FA, DeMayo FJ, Lydon JP, Tsai SY, Tsai MJ: Null mutation of mCOUP-TFI results in defects in morphogenesis of the glossopharyngeal ganglion, axonal projection, and arborization. Genes Dev 1997; 11: 1925–1937
59. Zhou C, Qiu Y, Pereira FA, Crair MC, Tsai SY, Tsai MJ: The nuclear orphan receptor COUP-TFI is required for differentiation of subplate neurons and guidance of thalamocortical axons. Neuron 1999; 24: 847–859
60. Brubaker K, McMillan M, Neuman T, Nornes HO: All-trans retinoic acid affects the expression of orphan receptors COUP-TFI and COUP-TFII in the developing neural tube. Brain Res Dev Brain Res 1996; 93: 198–202
61. Fjose A, Weber U, Mlodzik M: A novel vertebrate svp-related nuclear receptor is expressed as a step gradient in developing rhombomeres and is affected by retinoic acid. Mech Dev 1995; 52: 233–246
62. Jonk LJ, de Jonge ME, Pals CE, Wissink S, Vervaart JM, Schoorlemmer J, Kruijer W: Cloning and expression during development of three murine members of the COUP family of nuclear orphan receptors. Mech Dev 1994; 47: 81–97
63. van der Wees J, Matharu PJ, de Roos K, Destree OH, Godsave SF, Durston AJ, Sweeney GE: Developmental expression and differential regulation by retinoic acid of Xenopus COUP-TF-A and COUP-TF-B. Mech Dev 1996; 54: 173–184
64. Jones-Villeneuve EM, McBurney MW, Rogers KA, Kalnins VI: Retinoic acid induces embryonal carcinoma cells to differentiate into neurons and glial cells. J Cell Biol 1982; 94: 253–262
65. McBurney MW, Jones-Villeneuve EM, Edwards MK, Anderson PJ: Control of muscle and neuronal differentiation in a cultured embryonal carcinoma cell line. Nature 1982; 299: 165–167
66. Mummery CL, Feijen A, Moolenaar WH, van den Brink CE, de Laat SW: Establishment of a differentiated mesodermal line from P19 EC cells expressing functional PDGF and EGF receptors. Exp Cell Res 1986; 165: 229–242
67. Imai Y, Suzuki Y, Tohyama M, Wanaka A, Takagi T: Cloning and expression of a neural differentiation-associated gene, p205, in the embryonal carcinoma cell line P19 and in the developing mouse. Brain Res Mol Brain Res 1994; 24: 313–319
68. Imai Y, Suzuki Y, Matsui T, Tohyama M, Wanaka A, Takagi T: Cloning of a retinoic acid-induced gene, GT1, in the embryonal carcinoma cell line P19: neuron-specific expression in the mouse brain. Brain Res Mol Brain Res 1995; 31: 1–9

69. Jonk LJ, de Jonge ME, Vervaart JM, Wissink S, Kruijer W: Isolation and developmental expression of retinoic-acid-induced genes. Dev Biol 1994; 161: 604–614
70. Adam F, Sourisseau T, Metivier R, Le Page Y, Desbois C, Michel D, Salbert G: COUP-TFI (chicken ovalbumin upstream promoter-transcription factor I) regulates cell migration and axogenesis in differentiating P19 embryonal carcinoma cells. Mol Endocrinol 2000; 14: 1918–1933
71. Bishop KM, Goudreau G, O'Leary DD: Regulation of area identity in the mammalian neocortex by Emx2 and Pax6. Science 2000; 288: 344–349
72. Mallamaci A, Muzio L, Chan CH, Parnavelas J, Boncinelli E: Area identity shifts in the early cerebral cortex of Emx2−/− mutant mice. Nat Neurosci 2000; 3: 679–686
73. Zhou C, Tsai SY, Tsai MJ: COUP-TFI: an intrinsic factor for early regionalization of the neocortex. Genes Dev 2001; 15: 2054–2059
74. McGinnis W, Krumlauf R: Homeobox genes and axial patterning. Cell 1992; 68: 283–302
75. Horan GS, Ramirez-Solis R, Featherstone MS, Wolgemuth DJ, Bradley A, Behringer RR: Compound mutants for the paralogous hoxa-4, hoxb-4, and hoxd-4 genes show more complete homeotic transformations and a dose-dependent increase in the number of vertebrae transformed. Genes Dev 1995; 9: 1667–1677
76. Kessel M, Gruss P: Homeotic transformations of murine vertebrae and concomitant alteration of Hox codes induced by retinoic acid. Cell 1991; 67: 89–104
77. Le Y, Sauer B: Conditional gene knockout using cre recombinase. Methods Mol Biol 2000; 136: 477–485
78. Gu H, Marth JD, Orban PC, Mossmann H, Rajewsky K: Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting. Science 1994; 265: 103–106