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Review

COUP-TF orphan nuclear receptors in development and differentiation

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Abstract. Chicken ovalbumin upstream promoter transcription factors (COUP-TFs) are orphan members of the steroid/thyroid hormone receptor superfamily. They have been shown to negatively regulate the activation function of vitamin D, thyroid hormone, retinoic acid, the retinoid X and the peroxisome proliferator-activated receptors. COUP-TF genes have been cloned from many species and their sequences are exceptionally conserved through evolution. This suggests a critical role for the COUP-TFs in these organisms. Indeed, the *Drosophila* COUP-TF, *seven-up* and mouse COUP-TFII are essential for development and differentiation during embryogenesis. Our current understanding of COUP-TF function suggests that they serve vital physiological roles during development despite extensive overlaps of expression. This defines the COUP-TFs as important factors in regulation of development and differentiation in multiple organisms.

Key words. Orphan nuclear receptors; COUP-TF; embryogenesis.

COUP-TFs are Orphan Nuclear receptors

The steroid/thyroid hormone receptor superfamily of nuclear receptor proteins consists of many ligand-activated transcriptional regulators required in development, differentiation and homeostasis [1–6]. A large number of these proteins are orphan receptors whose ligands have yet to be identified [7]. Chicken ovalbumin upstream promoter-transcription-factors (COUP-TFs) are arguably one of the best-characterized orphan nuclear receptors (NR2F subgroup according to the nuclear receptor nomenclature, 1999) [8]. The first member, human COUP-TFI, was discovered as a transcription factor that bound the COUP element, which regulates transcription of the ovalbumin gene [9–13]. Independently, hCOUP-TFI was cloned as v-ErbA-related protein 3, EAR-3 [14], and subsequently a second human family member, hCOUP-TFII [15] was identified, which was also cloned as apolipoprotein regulating protein 1, ARP-1 [16].

Through homology screening, human COUP-TF homologs and orthologs have been obtained from numerous species, making the COUP-TF (NR2F) subgroup of orphan nuclear receptors the largest within the nuclear receptor superfamily [7, 8, 17]. Based on alignment of their putative ligand-binding domains (LBDs), vertebrate COUP-TFs can be subdivided into four groups [8]. Most of the higher vertebrates, from human to chicken, contain genes encoding two COUP-TF subfamily members, whereas zebrafish and *Xenopus* have three members, and insects and invertebrates such as *Drosophila*, *Caenorhabditis elegans* and the sea urchin contain only one member. Within a given subgroup, the homology in both the DNA binding domains (DBDs)

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and the putative LBDs are striking. The DBDs of COUP-TFI or -II in different species are virtually identical, implying that they bind to a similar if not identical response element [8]. Most surprisingly, the putative LBDs of COUP-TFI and -II are 99.6% identical among vertebrates and 90% between human and fly [8]. Such a high degree of sequence conservation suggests that these domains are critical for the biological function of COUP-TFs, although a ligand for COUP-TFs has not been identified. In contrast, the N-terminal domains of COUP-TFI and are significantly divergent, having only 45% identity, and -II may provide distinct functions for the two different members [11, 15, 16, 18].

Based on this striking sequence conservation over the millions of years of evolution, it is reasonable to speculate that COUP-TFs may play a vital role for cellular function. This hypothesis is supported by the finding that null mutants of *Drosophila svp* are lethal [19, 20] and COUP-TFI and -II loss-of-function mouse mutants also lead to perinatal and embryonic lethality, respectively [21, 22]. This review is intended as an update of our understanding of COUP-TF function in development and differentiation (for a cumulative review the reader is referred to [8]).

Biochemical characteristics and gene regulation

Biochemical studies indicate that COUP-TFs exist in solution as dimers and bind with high affinity to an imperfect direct repeat separated by one nucleotide (GTGTCAAAGGTCA, or DR1) [8, 23]. However, the ability of COUP-TFs to bind to a number of variably spaced direct repeats [23] suggests that COUP-TFs are able to assume different conformations to accommodate structural and spatial changes in the recognition sequences [23, 24]. Indeed, COUP-TF dimers bound to either a DR1 or DR6 element possess distinct conformations as shown by their different susceptibilities to protease digestion [23]. By virtue of their promiscuous DNA binding properties and the ability to compete for the same hormone response element as other nuclear receptors, COUP-TFs negatively regulate a large number of genes (table 1 and 2) [8, 23–26]. In addition, COUP-TFs form DNA-binding heterodimers with RXR, a universal heterodimeric partner of many nuclear receptors [23, 24, 26]. Therefore, COUP-TFs modulate the hormone responsiveness of a large number of nuclear receptors by reducing the availability of RXR [8]. Thus, in general, COUP-TFs have been considered as negative regulators in vivo. Like COUP-TFs, the *Drosophila* COUP-TFI homolog, *svp*, modulates the function of Ultraspiracle (*Usp*), the *Drosophila* homolog of RXR and the heterodimeric partner of the ecdysone receptor [27, 28]. Thus, *svp* negatively modulates the ecdysone signaling pathway in Drosophila in a manner similar to the modulation of thyroid hormone and retinoid function by COUP-TF in mammalian systems [28, 29]. Finally, COUP-TFs possess an active silencing domain which interacts with cellular corepressors, such as SMRT, N-CoR, Rip13 and other proteins yet to be identified, to silence both basal and active transcription of a variety of transactivators [30–33]. In addition to being repressors, the COUP-TFs can also activate an ever-growing list of gene promoters in vitro (table 2). Analysis of the mechanisms by which COUP-TFs positively regulate expression of target genes suggests that protein-protein interactions with known coactivators, such as p300 or other transcription factors, may be required for regulation of cell-specific differentiation pathways [33, 34].

The COUP-TFs have been molecularly characterized to regulate transcription of many promoters containing the DR1 element of genes involved in fat metabolism (table 1). These include several apolipoproteins for fatty acid transport in the blood, several enzymes involved in β-oxidation (mitochondrial and peroxisomal), and enzymes involved in fatty acid synthesis. Many of these gene promoters are also regulated by the peroxisome proliferator-activated nuclear receptors (PPARs) that bind to the same upstream responsive DR1 element (which are also known as a PPARE) through heterodimerization with the RXRs. Peroxisome proliferators form a family of diverse xenobiotic compounds that include hypolipidemic agents, herbicides and plasticizers. These compounds activate transcription of a subset of nuclear genes including those encoding peroxisomal fatty acid β-oxidation enzymes, whose elevated activities can lead to hepatocarcinogenesis. For example, induction of the genes encoding fatty acyl-CoA oxidase and enoyl-CoA hydratase-dehydrogenase, the first and second enzymes of the pathway, is mediated by PPARs. In vitro analyses have shown that COUP-TFs are able to repress transcription of the fatty acyl-CoA oxidase but activate the enoyl-CoA hydratase-dehydrogenase. Interestingly, COUP-TF can inhibit preadipocyte differentiation [35], and the *Drosophila svp* gene is required for fat cell differentiation (see below) [19]. However, it is not known what role the mammalian genes play in fat metabolism and fat cell differentiation in vivo.

Sonic hedgehog regulates the *COUP-TFII* gene, and a Shh-response element was identified in the COUP-TFII promoter. The Shh-response element binds to a factor distinct from Gli, a gene known to mediate Shh signaling. Although this binding activity is specifically stimulated by Shh-N (amino-terminal signaling domain), it can also be unmasked with protein phosphatase treatment in the mouse cell line P19, and induction by Shh-N can be blocked by phosphatase inhibitors. Thus,

Table 1. Genes inhibited by COUP-TF.

Target Gene	DNA site	Competing Factor	Tissue/cells	Gene Function	Reference
hErythropoietin	HRE	HNF4	HepG2	bone marrow stem cell differentiation	[51]
hFactor IX		HNF4	HepG2	clotting factor	[52]
hAntithrombin	HRE	HNF4, RXR	HepG2;BSC40	coagulation	[53]
m/r Hemopexin	DR0	HNF4	HepG2	heme/porphyrin binding transport/protein	[54]
mLactoferrin	ERE	ER		transport iron to erythrocytes; antibacterial	[55]
hTransferrin	promoter 1		Hep3B; Sertoli	iron transport	[56]
hApolipoprotein A-IA	PPARE(DR1)	HNF4	Liver	fatty acid transport in blood	[57]
Apolipoprotein A-II	PPARE(DR1)			fatty acid transport in blood	[16]
Apolipoprotein A-IV		HNF4	HepG2; Caco-2; HeLa cells		[58]
Apolipoprotein B	DR1			fatty acid transport in blood	[16]
Apolipoprotein C-II	TRE	TR	HepG2, Cos 1	fatty acid transport in blood	[59]
r/hApolipoprotein C-III	PPARE [DR1), DR0, DR5	HNF4, TR, PPAR	HepG2; Caco2	fatty acid and cholesterol transport in blood	[60–62]
cApolipoprotein VLDLII	ERE		hepatocytes	fatty acid transport in blood	[63, 64]
rInsulin II	DR6		HeLa	promotes glucose utilization, protein synthesis, neutral lipid formation/storage	[65, 66]
Malic enzyme	PPARE	PPAR/RXRα		fatty acid synthesis, NADPH production	[67]
hLong-chain Acyl-CoA dehydrogenase (LCAD) Ornithine transcarbamyolase (OTC)	PPARE(DR1)			fat metabolism urea cycle	[68] [69]
HMG-CoA Synthase	PPARE (DR1)	HNF4	Leydig tumor	ketone body synthesis cell line (R2C)	[70, 71]
Acyl-CoA Oxidase	PPARE (DR1)	PPAR, HNF4		peroxisomal β-oxidation	[61]
hEnoyl-CoA hydratase	PPARE(DR1)	HNF4		peroxisomal P-OxIdation A	[72]
Medium chain acyl-CoA dehydrogenase (MCAD)	ER8, ER14	HNF4		mitochondrial β-Oxidation	[73]
Cholesteryl ester transfer protein (CETP)	-300 promoter		HepG2;CaCo	synthesis of cholesterol esters	[74]
rTestosterone 6beta-hydroxylase (CYP3A1)		HNF4	HepG2	testosterone metabolism	[75]
rCytochrome P4503A23 (CYP3A)	DR1	HNF4		metabolism of exogenous compounds	[76]
mP450 aromatase	half-site and	SF1 cAMP-RE	endometrium	synthesis of estrogen	[77]
rSteroid 17 α monooxygenase (P450c17)			mouse adrenocortical-Y-Leydig MA-10	progesterone metabolism	[78]
bSteroid 17 α-hydroxylase (CYP17)	DR6	SF1	Sertoli	inhibit ACTH induction, steroid synthesis	[79]
hP450 2D6 (CYP2D6)	DR1	HNF4	HepG2, Cos7	oxidative metabolism of compunds	[80]
Osteocalcin	VDRE (DR3)			mineralization and calcium ion homeostasis	[81]
mHepatocyte growth factor (HGF)	ERE	ER	liver cells	cytokine for growth and differentiation	[82]
m/h Oxytocin	DR0	ER	P19 EC	myometrial contractions at term; promotes milk release during lactation	[83, 84]
mOct4	DR1	RARα/RXRα RARβ/RXRα RARβ/RXRβ	P19	germ, cell/EC cell differentiation	[85, 86]
mDax 1	DR1	SF1	JEG-3	organ development	[87]
Purkinje cell protein (PCP-2)	TRE	TR	Purkinje cell	inhibit T_3 action	[88]
rKainate-preferring glutamate receptor subunit KA2 (GRIK5)		NURR1	CV1, ratCG4	glutamate transport	[89]
Preproenkephalin A	DR1			endorphin	[90]

Table 1. (Continued)

Target Gene	DNA site	Competing Factor	Tissue/cells	Gene Function	Reference
mArrestin	DR7			retina differentiation	[91]
Retinoic acid iteceptor (RARβ)	βRARE(DR5)	RAR	human lung cells	inhibit RA action	[92]
hRetinoid X receptor (RXRγ2)	γRXRE(DR1)	RXR	CV1	inhibit RA action	[93]
hSheketal (α-actin	TRE (DR4)	TR/RXR	C2C12	contractile element	[94]
Myosiri heavy chain	TRE (DR4)	TR/RXR		contractile element	[81]
suActin (CyIIIb)	DR2		HeLa	cytoskeletal element	[95]
Hepatitis B virus 1	LEF	HNF4, RXR		hepatitis; inhibit pre-RNA transcription	[96, 97]
HIV1-LTR	RARE (DR9)	RAR/RXR		inhibits virus replication	[98]
MMTV	DR1	S300II, RXR		mammary tumors	[12]

Ile genes are arbitrarily grouped by function, e.g. transport and fat metabolism. Abbreviations: h, human; r, rat; m, mouse; v, vertebrate; su, sea urchin; c, chicken; FIRE, hormone responsive element; DR, direct repeat; ER, everted repeat; IR, inverted repent-4r97 v

Shh-N signaling may result in dephosphorylation of a target factor that is required for activation of COUP-TFII-, Islet1- and Gli-response element-dependent gene expression. This finding has added to the complexity of the Shh signaling pathway. The phosphatase that mediates this dephosphorylation in response to Shh-N treatment is PP2A or is a pharmacologically similar phosphatase. This particular response is channeled through a protein with DNA binding activity apparently unrelated to that of the Ci (Cubutus interruptus)/Gli family [36]. A similar protein phosphatase activity is also required in the phosphorylation and processing of Ci55 to its active form Ci75 [37]. This pharmacologic similarity suggests the possibility that a phosphatase capable of influencing the activity of more than one downstream transcription factor acts early within the Hh signaling cascade.

Both COUP-TF genes are regulated by retinoids in vitro during retinoid- induced differentiation of P19 EC cells [8, 25, 38], and overexpression of COUP-TFI results in blockade of retinoic acid (RA)-induced neuronal differentiation of teratocarcinoma PCC7 cells [39]. In vivo, retinoids induce COUP-TFs in zebrafish [40] and mouse [41] hindbrain. For example, the zebrafish gene *svp*[40] (a COUP-TFII homolog) is expressed in specific regional and segmental domains within the developing brain. During the early embryonic stages when hindbrain rhombomeres are formed, a segmental expression pattern is established as a step gradient, coinciding directly with the four anteriormost segments. This suggests a role in controlling rhombomere- specific expression of genes contributing to cell differentiation in the hindbrain. Treatment of zebrafish embryos with retinoic acid affects the *svp*[40] step gradient and causes an elimination of a regional expression domain in the retina [40]. In a similar manner, COUP-TFII is implicated to be important in determination of the dorso-ventral dimension of the vertebrate retina through regulation of retinoid signaling [42]. Taken together, these in vitro and in vivo observations are consistent with the COUP-TFs being an integral part of the retinoid signaling network during development and differentiation.

Expression Patterns During Development

The patterns of COUP-TF expression have been described in the mouse, chick, zebrafish, frog, *C. elegans and Drosophila* [8, 25, 43]. We showed that COUP-TFI and -II exhibit overlapping, but distinct, expression patterns in all three germ layers during mouse development, with high expression of COUP-TFI in the nervous system and of COUP-TFII in the mesenchyme of internal organs [25, 44]. Expression of COUP-TFI and -II is first detected postgastrulation at embryonic day 7.5 (E7.5) in the neural ectoderm, peaks between E10–12, and overall declines sharply before birth [22]. The expression of COUP-TFs in the developing central nervous system (CNS) suggests a role in patterning and segmentation of the brain [22, 44, 45]. Differential expression of the COUP-TFs is apparent in many developing regions in the brain. COUP-TFI is detected in premigratory and migratory neural crest cells (NCCs) at E8.5, whereas COUP-TFII is not [22]. In E13.5 mouse embryos, COUP-TFI is detected throughout the pallium (the future cortex), whereas COUP-TFII is restricted caudally. Expression of COUP-TFs within the diencephalic neuromeres is in a segment-restricted manner where both COUP-TFI and -II are highly expressed in the D1 (future ventral thalamus and hypothalamus as

defined by Figdor and Stern [46]), and low in the D3/D4 regions (pretectal region). Finally, whereas COUP-TFI is highly expressed in the D2 region (dorsal thalamus), COUP-TFII is not [22]. Differential expressions of COUP-TFI and -II are also detected in other neuronal regions, including the midbrain and the spinal cord at midgestation. COUP-TFI is expressed throughout the neural tube, whereas COUP-TFII expression is restricted to the motor neurons [44]. The differential COUP-TF expression patterns during mouse CNS development suggest that they may be required for neuronal development and differentiation.

In addition to neural ectoderm expression, COUP-TFs are also expressed in mesoderm and endoderm during organogenesis [25, 38]. COUP-TFII is expressed in the mesenchyme of the nasal septum, tongue, follicles of vibrissae and cochlea [25]. COUP-TFI is expressed in the same regions, but at a considerably higher level [25]. In contrast, COUP-TFII is expressed highly in the mesenchyme of the developing salivary gland, atrium of the heart, lung, stomach, pancreas primordium, mesonephros, kidney and prostate, whereas COUP-TFI is expressed at a much lower level in these tissues [25]. In general, organs that require mesenchymal differentiation to epithelium display expression of COUP-TFII in the mesenchyme, but not in the terminally differentiated epithelium. Conversely, organs that develop by epithelial proliferation and differentiation highly express COUP-TFI in the epithelial cells like the ectoderm of the inner ear and tooth or the endoderm of the lung buds. Collectively, these results support the hypothesis that COUP-TFs control the expression of signals required for epithelial differentiation.

Physiological functions in model organisms

Functions of *Drosophila svp*

The *Drosophila svp* (a COUP-TFI homolog) has been shown to specify photoreceptor subtype during develop-

Table 2. Genes activated by COUP-TF.

Target Gene	Element	Cofactor	Tissue	Gene Function	Reference
rtEstrogen receptor (ER)	Half-site and ERE		yeast	menstruation and bone maturation	[99]
mParanthyroid hormone/Parathyroid hormone-related peptide (PTH/PHrP)	DR1	RXRα		bone resorption	[100]
vHepatocyte nuclear factor 1 (HNF1)				endoderm differentiation	[101]
Nerve growth factor-induced protein (NGFI-A)	SP1	SP1	CV1	brain, organ and vascular development	[102]
RARβ	RARE	NGFI-B	lung cells	homeostatsis	[92]
Enoyl-CoA hydratase/dehydrogenase	PPARE	PPAR	yeast, HeLa	peroxisomal β-oxidation	[72, 103]
HMG-CoA synthase	GC-rich		HepG2	ketone body synthesis	[70]
Fatty acid-binding protein				transport of intracellular fatty acids	
r/h Cholesterol 7α hydroxylase (CYP7A)	DR1	HNF4, LXRα	HepG2, Cos1	rate-limiting enzyme in bile acid synthesis (cholesterol homeostasis)	[104–106]
Phosphoenoyl carboxy kinase (PEPCK)	AF-1	HNF4		key enzyme in gluconeogenesis; biosynthesis decreased by insulin; glycerogenesis (adipose tissue)	[107]
Cholesteryl ester-transfer protein (CETP)	-636 promotor		HepG2; CaCo	synthesis of cholesterol esters	[74]
Apolipoprotein CII	TRE	HNF4	HepG2; Cos1	fatty acid transport in blood	[59]
hTransferrin			Hep3B	iron ion transport	[56]
cOvalbumin	DR1	none	HeLa	egg protein	[10, 108]
rα-Fetoprotein (AFP)	HRE		HepG2	tumor and neural tube defect marker	[109]
hH1 (0)	DR8	TR, RXR		chromosome condensation	[110]
HIV1-LTR	Palindrome, IR9; SP1, SP3	TR4; SP1	CHO; microglial; oligodendroglioma cells	virus replication	[111–114]
MMTV	DR1			mammary tumors	[115]

ment of the compound eye. Ectopic expression of svp in cone cells converts the cone cells to possess neuronal identity, and ectopic expression in other photoreceptor subtypes maintains the neuronal characteristics but loses the specific subtype identity [20]. Therefore, *svp* acts as a cell fate switch with the specific phenotype depending upon the developmental stage of the ommatidium at the time of *svp* expression [47]. Interestingly, *svp* also plays a role in fat-body development [19]. SVP is expressed transiently within the fat-cell lineage from stage 12, the beginning of early fat-cell differentiation, to 14. Stage 12 *svp*-positive cells within the mesoderm are thought to be the early precursor fat cells and indeed, loss of *svp* function resulted in the loss of at least two terminal fat-cell differentiation genes. Thus, *svp* plays a role in fat-body-specific terminal differentiation. Finally, *svp* has been shown to be required for development of the *Drosophila* kidneys, the Malpighian tubules (MTs) [48]. The *Drosophila* MTs form a simple excretory epithelium comparable in function to kidneys in vertebrates. *Svp* is an essential component that becomes induced in response to mitogenic epidermal growth factor (EGF) receptor-signaling activity emanating from the tip cell. *Svp* in turn is capable of regulating the transcription of cell cycle regulators [48] in the tip cell, which is decisive for controlling the proliferation of its neighboring cells. In amorphic *svp* mutants, a reduction of the tubule cell number, as compared with wild type, suggests *svp* is an integral component of the network that regulates division in the cells that receive the mitogenic signal from the tip cell [48]. Thus, *svp* has been shown to be critical for photoreceptor cell fate determination, a regulator of cell cycle in MTs and for fat-body differentiation in *Drosophila*.

Overexpression in *Xenopus*

Misexpression of human COUP-TFI also has been shown to dramatically affect early *Xenopus* development [49]. Overexpression of COUP-TFI in the dorsal half, but not the ventral half of *Xenopus* embryos, led to alterations in anterior development. The abnormal early development may result from perturbation of anterior early gene transcription. In addition, overexpression of COUP-TFI also inhibits retinoid-induced expression of xliml and krox20 supporting the idea that COUP-TFs are negative-feedback regulators of the retinoid signaling pathway [49]. Whether ectopic COUP-TFI expression will cause similar anterior head developmental defects in higher vertebrates and emulates those observed in *Xenopus* has yet to be determined.

Function of *C. elegans unc-55*

Mutations in *unc*-55 (a COUP-TFII homolog) in *C. elegans* cause Ventral D. (VD) motor neurons to adopt the synaptic pattern Dorsal D (DD) motor neurons and result in an asymmetric locomotive pattern when the animals move backward [43]. UNC-55 is expressed in the VD but not the DD motor neurons. The sinuous forward and backward locomotion exhibited by *C. elegans* is produced by two neural circuits one dedicated to forward movement and the other dedicated to backward movement. These two circuits converge on the dorsal and ventral body wall muscles and on two classes of inhibitory motor neurons: six dorsal D b (DD) motor neurons (born embryonically) and 13 VD motor neurons (born postembryonically). Activated UNC-55 receptors are proposed to modify the expression of the common D motor neuron genetic program. Thus, the VD motor neuron proteins targeted for presynaptic and postsynaptic processes are not redirected, whereas similar DD motor neuron proteins are redirected, thereby creating the synaptic pattern that distinguishes the two related classes of motor neurons [43].

Function of mouse COUP-TFs

Loss-of-function COUP-TFII mouse mutants were generated by targeted recombination in ES cells [21]. Two-thirds of heterozygote COUP-TFII mice die before weaning, and homozygous deletion of the *COUP-TFII* gene is lethal around E10. Embryos are growth-retarded in the head and heart, and have severe hemorrhage and edema by E9.5. Histological analyses revealed enlarged blood vessels, a lack of normal development of the atria and sinus venosus, and malformed cardinal veins. Immunological and molecular analyses of the vascular system show a decrease in the extent and complexity of the microvasculature in the head and spine regions, suggesting that angiogenesis and vascular remodeling are defective in COUP-TFII mutants. These defects are consistent with a loss of COUP-TFII function in the mesenchymal compartments of the head, spine and heart. Analyses of multiple ligand-receptor tyrosine kinase pathways that regulate primitive vascular development revealed that Angiopietin-1 (Ang1) is downregulated in COUP-TFII mutants. Thus, perturbations of the Ang1–Tie2 receptor pathway are suggested to contribute to the heart and vasculature defects observed in COLIP-TFII mutants. This suggests that COUP-TFII may modulate embryonic heart and vasculature formation via mesenchymal-endothelial signaling. Interestingly, using subtraction library screening, Ang1 has been isolated several times as a target gene of COUP-TFII action [S. Y. Tsai, unpublished data]. Whether the regulation of Ang1 is direct or indirect is presently unknown and will require isolation and analysis of its promoter.

Loss-of-function COUP-TFII mouse mutants result in perinatal lethality. Ninety-five percent of newborn mutants have asymmetric fusions of the glossopharyngeal (IX) and vagus (X) cranial nerves [22]. The glossopharyngeal nerve innervates the stylopharyngeus muscle of the pharynx and carotid artery and body; it also sends taste and sensing fibers to the posterior one-third of the tongue. Defects in the glossopharyngeal nerve impair both sensory and motor functions of the pharynx and the tongue and compromise feeding behavior in COUP-TFI mutants, resulting in malnutrition, dehydration and usually perinatal death. Whole-mount in situ analysis for COUP-TFI transcripts discovered that it is a marker of premigratory and migratory neural crest cells in the hindbrain. Some neural crest precursors of the IX cranial neurons underwent apoptosis prior to formation of the ganglion, resulting in an aberrant formation of the superior component of the IX ganglion. Aside from cranial nerve fusions, arborization of axons is severely reduced in the cervical plexus region as well as in the ophthalmic branch of the trigeminal nerve. This significantly limited axonal arborization in COUP-TFI mutants in comparison to heterozygous or wild-type littermates contributed to the inability to feed, resulting in perinatal death of mutants [22]. The limited arborization was not due to a delay in development, since the same phenotypes were seen at different somite stages. Both phenotypic changes in the mutants suggest that COUP-TFI may modulate axon guidance. Whether the observable defects arise from the lack of guidance cues, the inability to sense the cues or both is not known at present.

COUP-TFI may also be a critical component in bone differentiation, as virtually all COUP-TFI mutants (98%) have a premature fusion of the left and/or the right exoccipital bones with the basioccipital bone [8]. These bones are derived from the occipital somites. Whole mount-mount in situ studies at E9 showed that COUP-TFI is abundantly expressed in the paraxial mesoderm of the anterior-most occipital somites. Thus, the observed occipital bone fusion in mutants is consistent with the idea that COUP-TFI plays a major role in the differentiation of these bones. Similar ossified fusions are observed in double-knockout mutant mice of the RARα1 and RARβ genes [50]. Since COUP-TFI is regulated by retinoids and is considered to be a downstream target of the retinoid signaling pathway [25, 38, 39], it is not surprising that mutation of either COUP-TFI or retinoic acid receptors (RARs) result in similar phenotypes. On the other hand, many other defects seen in the RAR double knockouts are not observed in COUP-TFI mutants [22, 50]. Whether this is due to partial functional redundancy between COUP-TFI and II, or limited convergence of the signaling pathways shared by COUP-TFI and the retinoids, is yet to be defined.

Finally, the cerebral cortex lamina structure in COUP-TFI mutants is defective where cortical layer IV is absent and this was due to excessive cell death [18]. The death of cortical layer IV neurons was the consequence of the failure of thalamocortical axons to project to their cortical targets and to innervate layer IV neurons in COUP-TFI mutant cortex. Moreover, subplate neurons underwent improper differentiation and premature cell death during corticogenesis. Improper differentiation of subplate neurons had been shown to lead to the altered expression of axonal guidance cues which are critical for guiding thalamocortical axons and proper cortical innervation. Thus, the lack of proper thalamocortical afferent inputs resulted in layer IV neuron cell death, culminating in the apparent absence of layer IV in the COUP-TFI mutant cortex. Thus, these findings demonstrate a critical role of the subplate in early corticothalamic connectivity and confirm the importance of afferent innervation for the survival of layer IV neurons. Taken together, these results also substantiate COUP-TFI as an important regulator of neuronal development and differentiation.

Perspectives

The data have identified that COUP-TFs play multiple roles, from cell fate determination to regulation of cell cycle, to maintenance of cell survival, to regulation of appropriate cell differentiation. Cumulatively, these data strongly suggest that COUP-TFs are critical for development and differentiation in many different tissues in several organisms. Further analyses of target genes regulated by COUP-TFs in vivo through analysis of the mutants will allow a better comprehension of the roles these orphan nuclear receptors play in development and differentiation.

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