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Chicken Ovalbumin Upstream Promoter Transcription Factor (COUP-TF): Expression During Mouse Embryogenesis

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Members of the steroid/thyroid hormone receptor superfamily such as TR, RAR, RXR and VDR are known to play important roles in regulation of gene expression during development, differentiation and homeostasis. COUP-TFs are orphan members of this superfamily of nuclear receptors and have been shown to negatively regulate the ability of these nuclear receptors to transactivate target genes. Two different mechanisms are implicated in this repression. First, COUP-TFs bind to AGGTCA direct repeats and palindromes with various spacings, which include response elements for TR, RAR, RXR and VDR, allowing for direct competition of COUP-TFs for the response elements. Second, COUP-TFs can heterodimerize with RXRs, the essential cofactor for effective binding of VDR, TRs and RARs to their cognate response elements. The physiological significance of this negative effect of COUP-TF on the activity of these receptors has been analyzed. Detection of COUP-TF transcripts during mouse development reveal discrete spatial and temporal expression domains consistent with COUP-TFs being involved in regulation of gene expression during embryogenesis. Transcripts are localized within discrete regions of the central and peripheral nervous system including the inner ear. In addition, COUP-TFs are found in many tissues including testes, ovary, prostate, skin, kidney, lung, stomach, intestine, pancreas and salivary gland. Some of these expression domains colocalize with those of TR, RAR, and RXR. The simultaneous expresson of these genes raise the possibility that COUP-TFs can act as negative regulatory factors during development and differentiation.

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THE COUP-TFs ARE ORPHAN NUCLEAR RECEPTORS

The steroid/thyroid hormone receptor superfamily of proteins comprise a large group of ligand-activated regulators of gene expression. Prototypic members of this nuclear receptor superfamily structurally consist of: (i) a variable N-terminal domain (A/B region) involved in gene activation and promoter selection; (ii) a highly conserved DNA binding domain (DBD, C region) with two type II zinc fingers (C4–C5); (iii) a short hinge region (D region); and (iv) a less conserved C-terminal domain (LBD, E/F region) which mediates ligand binding, dimerization, nuclear localization, and transactivation [1–4]. There are three regions (region I in the DBD, and regions II and III in the LBD) that show high homology within the group and which are distinctive of this superfamily.

Chicken ovalbumin upstream promoter transcription factors (COUP-TFs) were first identified to bind to direct repeats in the upstream promoter of the ovalbumin gene [5, 6] that conferred efficient activation of transcription on the ovalbumin gene *in vitro*. hCOUP-TFI has since been cloned from HeLa cells [7] but was independently cloned via homology as an erbA related protein 3 (EAR-3) without function [8]. Subsequently, hCOUP-TFII was cloned due to its high homology to hCOUP-TFI [9]. This protein is homologous to the apolipoprotein AI regulatory protein I (ARP-1) [10]. Analysis of the nucleotide and primary structure revealed that the COUP-TFs belong to the nuclear receptor superfamily [7–11]. No ligand has yet been identified for the COUP-TFs, thus, classifying them as orphan members of the nuclear receptor superfamily. COUP-TFs are the most studied among the orphan

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nuclear receptors due to their ability to regulate the activity of other members of this superfamily.

hCOUP-TFI homologs have been cloned from many species: *seven-up* (*svp*) from *Drosophilia* [12]; Sp-COUP-TF from sea urchin [13]; xCOUP-TF from *Xenopus* [14]; bCOUP-TFI from bovine [15]; *svp*[44] from zebrafish [16]; haCOUP-TF from hamster [M-J Tsai, unpublished]; and mCOUP-TFI from mouse [17, 18]. hCOUP-TFII homologs have also been cloned from bovine [15], chick [19], and from mouse [17, 18]. One distinguishing feature of the COUP-TFs is the extremely high homology between homologs [17, for review see 20]. The most striking example is that the human COUP-TFI primary sequence is identical to the murine and has greater than 99% identity with the hamster and *Drosophilia* proteins within the DBD and LBD. This low evolutionary divergence may point to the conserved importance of these domains within the COUP-TF group of nuclear receptors. Recently, the mEAR-2 gene has been proposed to be a third COUP-TF member on the basis of sequence homology and its ability to repress retinoic acid dependent activation of target genes [18, 21]. However, due to the lower sequence identity, the mEAR-2 gene is probably a more distant member of this group.

REGULATION OF GENE EXPRESSION BY COUP-TFs

COUP-TFs bind to a wide spectrum of response elements encompassing AGGTCA direct repeats and palindromes with various spacings [22], however, they have the highest affinity for a direct repeat of AGGTCA with one nucleotide spacing (DR1 element) [22–25]. This diverse range of DNA-binding specificities is presumed to be due to a vast structural adaptation of the COUP-TF proteins. Consequently, COUP-TFs are capable of binding to a variety of hormone response elements recognized by receptors for retinoic acid (RAR), retinoid X (RXR), thyroid hormone (TR), estrogen (ER), vitamin D3 (VDR), and the peroxisome proliferator-activated nuclear receptors (PPAR). Thus, this promiscuous binding to various response elements results in the inhibition of the transcriptional activity of ER, TR, VDR, RAR, PPAR, and RXR [22, 24–27]. Different mechanisms are implicated in this repression including: (i) direct competition of COUP-TFs for the active hormone response elements, which may result in silencing of basal transcriptional activity; and (ii) heterodimerization with RXRs, the essential cofactor for effective binding and functional activity of TR, VDR, RAR and PPAR [10, 22, 25–28]. Heterodimers have a much higher DNA-binding affinity than do homodimers and also exert maximal hormone-dependent transcriptional activity. Thus, the effective concentration of RXRs is reduced, resulting in indirect inhibition of the activation functions of RAR, TR, VDR and PPAR [26].

COUP-TFs have been shown to bind to promoter elements and negatively regulate a diverse assortment of genes. These include: hepatitis B virus (HBV) enhancer I [29]; *cis*-acting elements of apolipoprotein AI (ApoI), AII (ApoAII), B (ApoB), and CIII (ApoC-III) [21, 30–32] and AIV (ApoAIV) [33]; transferrin (Tf) gene in Sertoli cells [34]; L-pyruvate kinase (LPK) promoter [35]; ornithine transcarbamylase (OTC) promoter [36]; cellular retinol binding protein II (CRBP II) [37]; and medium chain acyl-CoA dehydrogenase (MCAD) [38]. The COUP-TF binding elements within these promoters have also been shown to be capable of binding the orphan receptor hepatocyte nuclear factor 4 (HNF-4). Upon binding, COUP-TFs repress whereas HNF-4 activates expression of these genes. Furthermore, the level of expression from these promoters depends on the ratio of the two factors, implicating that the inhibitory effect of COUP-TFs are due to direct competition with HNF-4 for binding to the common site [21].

In other gene contexts, COUP-TFs have been shown to bind and regulate expression in a promoter specific manner by hindering the binding of specific regulators to overlapping sites. COUP-TFs bind to the mouse lactoferrin estrogen response module repressing ER stimulation [39–41]. Each response element is functional when either factor is present alone. However, when both factors are present, an increasing amount of COUP-TFs effectively repress the activation function of ER. Mutations in the COUP-TF binding site relieve the inhibition. Therefore, COUP-TF may regulate the expression of lactoferrin in conjunction with estrogens.

COUP-TFs have been shown to regulate several other genes in a similar manner. COUP-TF binds to an overlapping response element in the oxytocin promoter repressing induction by steroidogenic factor I (SF-I) [42] and by estrogens, thyroid hormone and retinoic acid [43]. COUP-TF binds an element in: (i) the α-fetoprotein gene and represses transactivation by RXR [44]; (ii) the fatty acid acyl-CoA hydratase-dehydrogenase gene and antagonizes PPAR activation [45]; (iii) the R2 element in the class I major histocompatibility complex (MHC) promoter in Ad12-transformed cells and down-regulates its transcription [46]; and (iv) the negative glucocorticoid receptor element (nGRE) in the pro-opiomelanocortin gene avoiding repression by glucocorticoids [47–49]. In addition, COUP-TF has been shown to bind to the chicken ovalbumin gene [50], rat insulin II gene [51, 52], the apolipoprotein VLDL II promoter [53–55], the human immunodeficiency type 1 LTR [56], hepatocyte growth factor promoter [57], the hemopexin gene [58], and Oct-4 gene [59, 60].

COUP-TF binding to some response elements results in a silencing activity, repressing transcription below basal levels [26]. The physiological significance of this silencing activity is exemplified by the studies of

the regulation of Oct-4 gene in differentiating embryonal carcinoma (EC) cells [59]. Expression of transcription factor Oct-4 is associated with undifferentiated embryonic stem cells and primordial germ cells and is down-regulated during retinoic acid induced differentiation [61]. This retinoic acid induced down-regulation is conveyed through a negative element in the Oct-4 promoter which specifically binds COUP-TFs [62]. During retinoic acid induced differentiation, COUP-TFs are induced in EC cells and can bind to and silence the expression of Oct-4. Thus, COUP-TFs can singularly silence the expression of an otherwise active promoter.

Finally, COUP-TFs are capable of positively regulating gene expression *in vitro*. These include: the transferrin gene which is stimulated by COUP-TFI in concert with HNF-4 in liver and HeLa cells [34]; the intestinal fatty acid binding protein (Fabpi) promoter is induced in CaCo cells [63]; and COUP-TFI can stimulate the ornithine transcarbamylase and SV40 promoters in liver cells [36]. The overall significance of the positive regulation is not known as the stimulation is minimal (2–3-fold) and only demonstrated when the response elements are taken out of the endogenous promoter context.

COUP-TF EXPRESSION DURING DEVELOPMENT

Thyroid hormone and vitamin A derivatives are required for development, differentiation and growth in a wide variety of species [64, 65]. Since COUP-TFs have been shown to regulate the activity of these receptors, COUP-TFs may play important roles during development and differentiation. Indeed, the expression patterns of members of the COUP-TF family of transcription factors during development support this hypothesis [12, 16–20]. The *Drosophilia svp* gene is required for normal neurogenesis since homozygous null mutants are lethal [12]. Mosaic analyses identified *svp* to be required for normal development and differentiation of photoreceptor cells and may play a central role in their cell fate determination. Subsequent analysis of zebrafish *svp*[44] transcripts by *in situ* hybridization revealed further support of the results from *Drosophilia*, that COUP-TFs are crucial factors required for normal neurogenesis [16]. In addition, cCOUP-TFII is expressed transiently in chick spinal motor neurons and ectopic expression can be induced by a notochord graft [19]. This suggests that transcriptional regulation by cCOUP-TFII is downstream of inductive events emanating from the notochord and floorplate. Finally, the analysis of murine COUP-TF genes has further confirmed the importance of these transcription factors in neuronal development and differentiation, and are implicated in the specification of the diencephalic neuromeric compartments [17].

Apart from neuronal expression, COUP-TFs are also differentially expressed in a restricted manner during organogenesis. For example, mCOUP-TFI transcripts are abundantly expressed within the stroma of the nasal septum but not in the olfactory epithelium, in the cells surrounding the whisker follicles (follicles of vibrissae), and in the tongue of a 15.5 days post coitum (dpc) mouse embryo (Fig. 1). mCOUP-TFII transcripts are found in these same regions but at a lower level. Furthermore, at this same stage of gestation, mCOUP-TFII is highly expressed in the submandibular gland, lung, esophagus, stomach, pancreas primordium, mesonephros, testes, ovary (not shown), prostate (not shown), retina (not shown), limb bud (not shown), skin (not shown), and inner ear (not shown). Within the definitive kidney (metanephros), mCOUP-TFII transcripts are detected in the kidney capsule lining the kidney and the adrenal gland, in the stroma of the developing metanephros and Bowman's capsule but not in the collecting tubules nor in the adrenal gland. Jonk *et al.* [18] have shown a similar pattern of expression, however, there are several discrepancies compared to our analysis. They detect expression of mCOUP-TFI in the heart at 11.5 dpc and in the olfactory epithelium by 14.5 dpc. They also found mCOUP-TFII transcripts in the olfactory epithelium and in the adrenal gland at 14.5 dpc. Throughout our analyses none of these tissues were ever detected to have any mCOUP-TF transcripts even at earlier stages of gestation. These discrepancies may be attributed to differences in the stringency of the hybridization protocols. Transgenic analyses with the COUP-TF promoters driving reporter constructs will be able to resolve these discrepancies.

FUTURE PERSPECTIVES

Cumulatively, COUP-TFs are expressed in a spatial and temporal manner during development of a number of species. COUP-TFs are initially expressed in all the three germ layers and subsequently in some but not all their derivatives. COUP-TFs seem to be sequentially down-regulated to a basal level when differentiation of a specific organ is completed. Thus, are the COUP-TFs required for the specification of specific cell lineages or for the maintenance of the specific cell type which has been determined by upstream factors? On the contrary, are the COUP-TFs solely required for normal differentiation of a specific germ layer and are subsequently down-regulated? COUP-TFs have been found to regulate many liver specific genes *in vitro* yet the expression of COUP-TFs during liver development is only detected at basal levels argues for a maintenance role for COUP-TFs in the liver. On the other hand, COUP-TFs have been implicated in: (i) a cell fate determination of *Drosophilia* photoreceptor cells; (ii) the development of chicken motor neurons; and (iii) specification of diencephalic neuromeres in

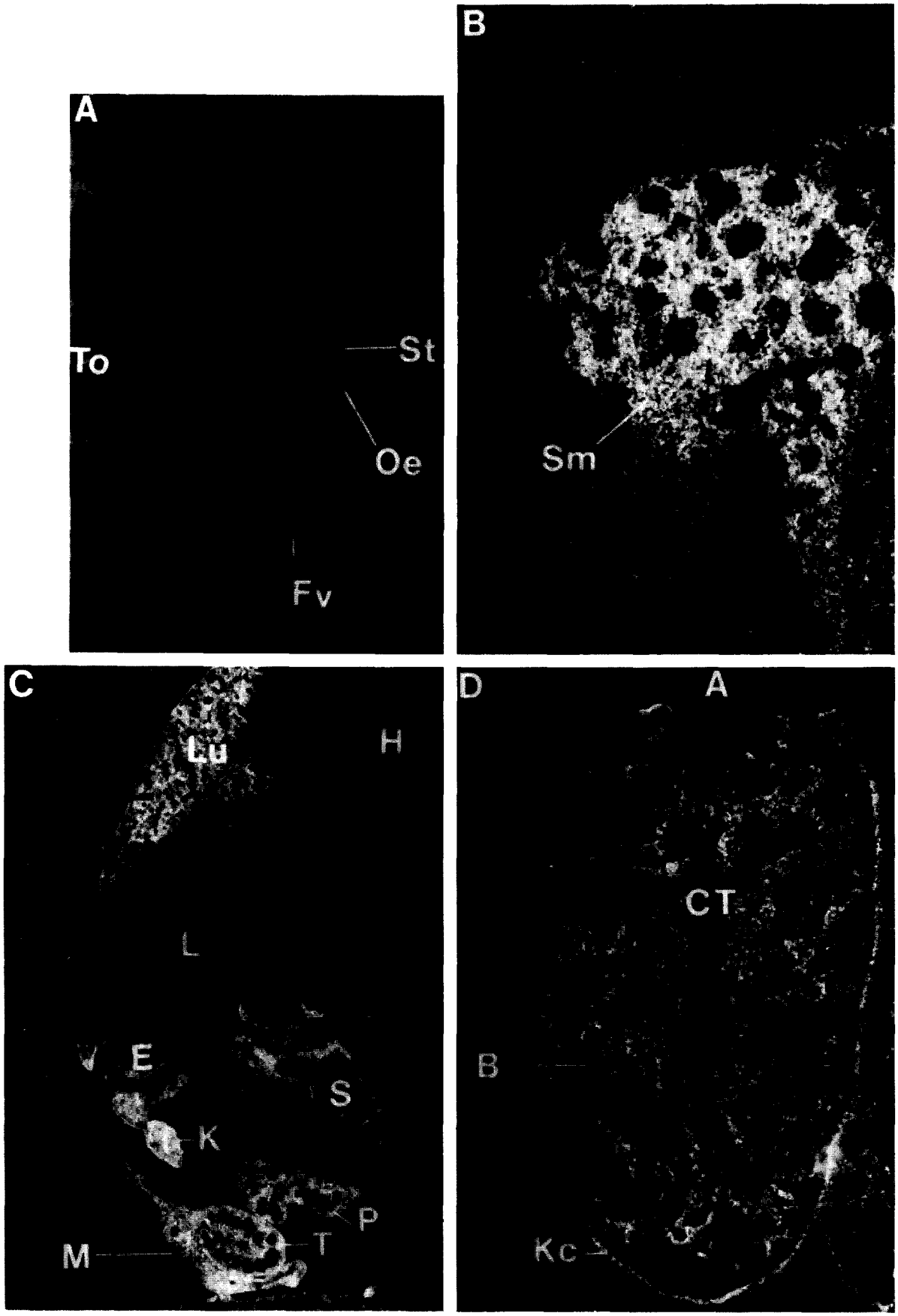

Fig. 1. Expression of mCOUP-TFs in a 15.5 dpc mouse embryo (A) mCOUP-TFI expression in a cross-section through the snout. (B) mCOUP-TFII expression in a sagittal section through the submandibular gland (Sm). (C) mCOUP-TFII expression in a parasagittal section through the embryo. (D) mCOUP-TFII expression in a sagittal section through the metanephros. Tongue (To), follicles of vibrissae (Fv), olfactory epithelium (Oe), nasal septum (St), lung (Lu), liver (L), heart (H), esophagus (E), stomach (S), kidney (K), pancreas primordium (P), testes (T) mesonephros and urogenital ridge (M), adrenal gland (A), collecting tubules (Ct), Bowman's capsule (B), and kidney capsule (Kc).

the mouse during embryonic development. Recently, COUP-TFI has been shown to be induced upon RA treatment of P19 embryonal carcinoma cells [18, 59, 66]. This implies that during embryogenesis, RA activates at least two pathways: one that activates RAR/RXR genes which in turn acts on retinoid specific target genes, and a negative feedback system that represses RAR/RXR mediated transactivation of target genes [66]. The interplay of members of the steroid/thyroid hormone receptor superfamily with the COUP-TFs brings another level of complexity to the role of COUP-TFs during development and differentiation. Analysis of the homozygous null mutants for COUP-TFs will shed more light on the importance of the COUP-TFs during development.

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