Published in final edited form as:

Biochim Biophys Acta. 2015 February ; 1849(2): 201–209. doi:10.1016/j.bbagr.2014.05.022.

COUP-TFs and Eye Development

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Abstract

Recent studies reveal that *COUP-TF* genes are essential for neural development, cardiovascular development, energy metabolism and adipogenesis, as well as for organogenesis of multiple systems. In this review, we mainly describe the *COUP-TF* genes, molecular mechanisms of COUP-TF action, and their crucial functions in the morphogenesis of the murine eye. Mutations of *COUP-TF* genes lead to the congenital coloboma and/or optic atrophy in both mouse and human, indicating that the study on COUP-TFs and the eye will benefit our understanding of the etiology of human ocular diseases. This article is part of a Special Issue entitled: Nuclear receptors in animal development.

Keywords

COUP-TFI; COUP-TFII; Eye; Development

1. Introduction

COUP-TFs were named Chicken Ovalbumin Upstream Promoter Transcriptional Factors, since they bind specifically to the promoter region of the chicken ovalbumin gene and regulate its expression. COUP-TF protein was originally purified from HeLa cell nuclear extracts and homogenous chicken oviduct cells, respectively [1–3]. The first cDNA clone encoding COUP-TF protein was isolated from a HeLa cell cDNA library, and subsequent sequencing revealed that COUP-TF is a member of the steroid/thyroid hormone receptor superfamily [4]. In mammals, there are two COUP-TFs, COUP-TFI (also known as EAR-3, NR2F1, according to the Nuclear Receptors Nomenclature Committee 1999) [4, 5] and COUP-TFII (also known as ARP-1, NR2F2) [6–8]. COUP-TFs are widely but not ubiquitously expressed in the developing mouse embryo. Our studies and others have

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demonstrated that COUP-TFs are essential for the neural development of the periphery nervous system (PNS) and the central nervous system (CNS), including the cerebrum, cerebellum and eye [9–21]; cardiovascular development such as angiogenesis, lymphangiogenesis, heart development, specification of vein and coronary vessel identity [22–27]; energy metabolism and adipogenesis [28–30]; as well as organogenesis of the urogenital/reproductive system, stomach, kidney, limb and skeletal muscle [31–38].

The eye, a specialized sub-region in the CNS, has been used as an excellent model to study the patterning, regionalization, and cell fate commitment for more than a hundred years. Eye morphogenesis is a multi-step process, including formation of the eye field, optic pit, optic vesicle, optic cup, lens, and neurogenesis in the neural retina. Many intracellular factors and extracellular signals participate in the sequential events during ocular development to ensure the appropriate formation and maturation of the eye [39, 40].

The expression and functions of the *COUP-TFI* gene in neurodevelopment, the *COUP-TFII* gene in various developmental processes and diseases, and *COUP-TF* genes in stem cells have been recently reviewed [41–43]. This review will mainly focus on the *COUP-TF* genes and their crucial roles in the morphogenesis of the murine eye, especially of the optic cup.

2. COUP-TF genes, proteins and molecular mechanisms of action

2.1. COUP-TF genes cloned from different organisms

In humans, *COUP-TFI* and *-TFII* genes are located at chromosome 5 and chromosome 15, respectively. Since the identification of human *COUP-TF* genes [2–8], their homologs have been cloned from many other organisms, including *mCOUP-TFI* and *-TFII* from mice [44, 45], *rCOUP-TFI* from rats [46], *xCOUP-TFA*, *-TFB* and *-TFC* from *Xenopus* [47–49], *cCOUP-TFII* from chicken [50], *bCOUP-TFI* and *-TFII* from bovine [51], the *seven-up* gene (*svp*) from *Drosophila* [52], the *svp*[40], *svp*[44] and *svp*[46] genes from zebrafish [53, 54], *spCOUP-TF* from sea urchin [55], and *AaSvp* in mosquito *Aedes aegypti* [56]. Their sequences reveal that *COUP-TF* genes are highly conserved from human to invertebrate [49].

2.2. COUP-TF proteins

COUP-TF proteins belong to orphan nuclear receptors, since the natural ligands of them have yet to be identified. As other classic nuclear receptors, COUP-TFs possess two highly conserved domains, the DNA binding domain (DBD) and ligand binding domain (LBD). The DBD domain of COUP-TFs contains 66 amino acids forming two conserved Zn-finger motifs. Human COUP-TFI and -TFII are 98% identical in the DBD region. Furthermore, the DBDs of COUP-TFs in different organisms are almost identical, highly indicating that they bind to similar *cis*-responding elements [57]. COUP-TFs bind to directed, inverted, and everted repeats of the consensus sequence, and show the relatively highest binding affinity for the imperfect direct repeat of AGGTCA separated by one nucleotide (DR1), such as GTGTCA A AGGTCA in the ovalbumin promoter [1, 58, 59]. The LBD domain of COUP-TFs contains two regions, which are important for the formation of a ligand-binding pocket [60]. The two regions of COUP-TF LBD are 96% and 100% identical. COUP-TFs could form heterodimers with RAR, TR, and RXR [61–64].

2.3. Molecular mechanisms of COUP-TFs action

COUP-TFs function not only as activators to promote gene expression, but also as repressors to inhibit gene expression. Accumulating studies *in vitro* and *in vivo* have demonstrated that COUP-TFs regulate the expression of their downstream target genes through two major molecular and cellular mechanisms.

2.3.1. COUP-TFs repress gene expression by directly binding to the DR site of the target genes—

Members of the steroid-thyroid hormone receptor superfamily regulate gene transcription through direct binding to discrete *cis*-elements [65–68]. Three amino acids at the C-terminal of the first zinc finger domain (known as the P box) of these receptors are essential to recognize the half-site nucleotide sequence. Based on the P-box sequence, COUP-TFs are classified as members of the ER-TR subfamily, which bind to the AGGTCA repeat [58, 59, 69, 70]. EMSA analysis reveals that COUP-TF forms a stable dimer to bind to the direct and palindromic repeats of GTCA motifs with variable spacing. Consequently, the DR1 consensus sequence is the most common COUP-TF element found in natural promoters [49]. DR elements with variable spacing have been identified from a number of COUP-TF target genes, such as *Pax6* in the eye [19, 27, 28, 30, 55, 70–95] (Fig 1A; Table 1). When COUP-TFs bind to the DR sequences, an active repression domain within putative COUP-TF LBDs could recruit transcriptional corepressors such as N-CoR, SMRT and histone deacetylase activities to the DNA to mediate gene silencing [96–99]. Thus, COUP-TFs repress gene expression by directly binding to the DR elements. Moreover, transient transfections and CAT assays show further that the activation of DR3, DR4 or DR5 containing reporters induced by VDR, TR, and RAR is inhibited in the presence of COUP-TF [59]. Thus, COUP-TFs can repress gene expression either by active repression or by competition for binding sites of other transcription factors.

2.3.2. COUP-TFs activate gene expression through tethering to the Sp1 transcription factor, which binds at the Sp1 site of the target genes—

In order to investigate the function of COUP-TFs in the development of the prostate, we generated several *COUP-TFI*-overexpressing rat urogenital mesenchymal (rUGM) cell lines, in which the elevated expression of *NGFI-A* transcripts and protein was observed. A 19 bp *cis*-element located between positions –64 and –46 in the *NGFI-A* promoter, which possesses two imperfect binding sites of the Sp1 family of transcription factors, is identified in response to the induction of both COUP-TFI and -TFII by band shift assay and promoter activity analysis. It is the Sp1 family of transcription factors but not the COUP-TF that directly interacts with the responsive element in the *NGFI-A* promoter in gel shift assays. The transactivation of the *NGFI-A* promoter by COUP-TF can be enhanced by coactivator p300 and steroid receptor coactivator 1. Furthermore, GST pull-down analysis reveals that COUP-TFI and Sp1 physically interact *in vitro* [100]. Accumulating evidence supports that COUP-TFs activate gene expression through tethering to the Sp1 transcription factor, which binds at the Sp1 site of the target genes, such as *Otx2* in the eye [16, 19, 20, 24, 27, 36, 71, 73, 100–102] (Fig 1B; Table 2). Consistent with this conclusion, chromatin immunoprecipitation assay (ChIP) indicates that COUP-TF is recruited to the Sp1 site and knockdown of *Sp1* or mutation of the Sp1 binding site eliminates the recruitment of COUP-TF to the Sp1 site [16, 71, 73, 100]

COUP-TFs are orphan members of the nuclear receptor superfamily. One of our recent studies reveals that in the absence of ligands, crystallized COUP-TFII LBD displays an autorepressed conformation, and the active COUP-TFII model possesses a ligand-binding pocket, which was further demonstrated by Eric Xu's laboratory that this pocket can be accessed by a steroid or retinoid ligand [103]. They demonstrated that COUP-TFII is activated by both 9-cis-retinoic acid and all-trans-retinoic acid in a dose dependent manner, indicating that probably COUP-TFII is a ligand-regulated nuclear receptor. Since the high doses of RAs are required for the activation of COUP-TFII, the physiological ligands of COUP-TF are likely to be factors other than RAs [103]. The identification of naïve ligands of COUP-TFs will broaden our understanding of working mechanisms of their action.

3. Eye development

3.1. Eye field, optic vesicle and optic cup

3.1.1. From early eye field to optic vesicle—In vertebrate animals, shortly after neural induction, a single eye field is specified in the medial anterior neural plate, where several highly conserved transcription factors such as Rx, Pax6 and Six3 are expressed [104]. Recent studies in both rodent and humans demonstrate that the single eye field is divided into bilateral hemispheres through regulations related to *Shh* and *Six3* genes [105–107]. However, so far the detailed molecular mechanisms to split the eye field are still largely unclear.

In mice, the first morphological sign of eye development is observed at around embryonic day (E) 8.0, with the presence of bilateral optic primordia, termed optic pits or optic sulci, in the presumptive ventral forebrain [108, 109]. Then the evagination of optic pits causes the formation of the early optic vesicle at about E9.5. Along the dorso-distal and ventro-proximal axis, the early optic vesicle is comprised of four main subregions, presumptive dorsal optic stalk (pdOS), presumptive retinal pigmented epithelium (pRPE), presumptive neural retina (pNR) and presumptive ventral optic stalk (pvOS). Meanwhile, the surface ectoderm thickens to form a lens placode, which interacts closely with the underlying optic vesicle for further morphological development of the eye [110, 111].

3.1.2. Optic cup, optic fissure and coloboma

Following the evagination, an invagination event takes place in both the optic vesicle and lens placode, resulting in the formation of a lens vesicle and a dual-layered optic cup at around E10.5. The optic cup is comprised of three main domains: the neural retina (NR), retinal pigmented epithelium (RPE), and optic stalk (OS) [39]. From then on, the morphology of the eye is patterned and maintained to adulthood. Along the disto-proximal axis, the invagination generates a groove at the ventral edge of the optic cup from the surface of the neural retina to the optic stalk. Fast growing ends of the optic cup contact and fuse at the optic fissure. The optic fissure is closed completely at E13.5, and if the optic fissure fails to fuse, the phenotype of coloboma, a human congenital ocular disease, would be generated [112].

3.2. Neurogenesis in the neural retina

The neural retina is a relatively simple neural tissue, and it has been widely used to study cell fate commitment, specification and differentiation in the CNS. The mature murine neural retina is comprised of three cell layers, the outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL). There are six neuronal cell types such as the rod, cone, horizontal, bipolar, amacrine and ganglion cell, and one glial cell type, the Müller cell. The nuclei of rod photoreceptor and cone photoreceptor cells are localized in the ONL, the nuclei of bipolar, amacrine, horizontal and Müller glial cells are in the INL, and the nuclei of retinal ganglion cells and displaced amacrine cells are in the GCL. The multipotential neural retina progenitor cells are specified at the early optic cup stage, following the expression of *Vsx2* [113–116]. Then, the neurogenesis of neural retina progenitor cells progress in an ordered pattern, with the differentiation of retinal ganglion cells and horizontal cells first, then cone-photoreceptors, amacrine cells, rod-photoreceptors, bipolar cells and, last, Müller glial cells [117, 118].

4. COUP-TF genes and eye development in the mouse

4.1. Expression of COUP-TFs during eye development

4.1.1. Expression of COUP-TFs in the optic vesicle—In the early murine optic vesicle at E9.5, along the dorso-distal and ventro-proximal axis, COUP-TFI and COUP-TFII are expressed highest in the pdOS and highly in the pRPE; whereas COUP-TFI but not COUP-TFII is expressed in the pNR and pvOS at relatively low levels. The expression of COUP-TFI is detected throughout the presumptive optic stalk at E9.5, and the expression of COUP-TFII is barely detected in the pvOS. Meanwhile, COUP-TFI also generates a temporal high- nasal low gradient at the distal plate of the early optic vesicle [17, 19].

4.1.2. Expression of COUP-TFs in the optic cup—The optic cup is formed around E10.5 after the initiation of invagination. COUP-TFI is expressed in the neural retina with a dorsal low-ventral high gradient, and COUP-TFII is markedly expressed in the single layered RPE. At the proximal optic stalk, the expression patterns of COUP-TFs remain similar as those at E9.5, with pan-optic stalk expression of COUP-TFI and dorsal optic stalk expression of COUP-TFII. At E11.5, a two layer-structure is observed at the optic stalk region, in which the expression of both COUP-TFI and -TFII is sharply reduced at the outer dorsal optic stalk (dOS), and high expression of COUP-TFI is maintained at the inner ventral optic stalk (vOS). At the same stage, COUP-TFI is mainly expressed in the neural retina with the highest level at the optic fissure; and COUP-TFII is exclusively expressed in the RPE and the dorsal neural retina. At E14.5, the expression profiles of COUP-TFI and -TFII are similar to those at E11.5, except for the expression of COUP-TFII in some amacrine cells [17, 19].

4.1.3. Expression of COUP-TFs in postnatal neural retina—The differential expression patterns of COUP-TFI ventral-high and COUP-TFII dorsal-high along the neural retina persists to adulthood. After birth, expression of COUP-TFI is detected in some cells in the INL and GCL. COUP-TFII expression is readily detected in amacrine cells in the INL [17](Tang, Tsai and Tsai, unpublished observations).

The dynamic expression profiles of COUP-TF genes in the early optic vesicle, optic cup, and neural retina strongly suggest that they play essential roles to program the appropriate morphogenesis of the murine eye.

4.2. Functions of COUP-TF genes in the eye development of the mouse

4.2.1. Function of COUP-TF genes in the formation of RPE—The progenitor cells in pRPE are bi-potent, capable of generating either RPE or NR identity. *Mitf*, a basic Helix-Loop-Helix-zip transcription factor, is not only a RPE marker, but also a determinant of the RPE fate. In several mutant mouse models of the *Mitf* gene, the RPE is transdifferentiated into NR [111, 119], and *Mitf* might antagonize *Vsx2* to specify RPE [114–116]. *Pax2* and *Pax6* genes, as well as *Otx1/2* genes are also involved in the RPE differentiation through regulating the expression of *Mitf* in the early optic vesicle [120, 121]. The expression of both COUP-TFI and -TFII is high in the pRPE at E9.5 [17, 19], indicating that they might play a critical role in the differentiation of the RPE. However, neither *COUP-TFI* nor *-TFII* single gene eye specific knockout mice show obvious RPE defects [19], suggesting that *COUP-TFI* and *-TFII* may compensate for each other during the differentiation of the RPE. Indeed, a secondary NR is observed at the prospective RPE region in the eye specific *COUP-TFI/-TFII* double knockout mouse embryos. The expression of *Mitf* and *Otx2* is markedly reduced in the prospective mutant ventral RPE region at E10.5. Meanwhile, the expression of *Pax6* is sharply increased in the RPE, accompanying the ectopic expression of *Vsx2* in some ventral RPE cells of the double mutants [19]. ChIP and real-time PCR assays in human ARPE-19 cells revealed further that COUP-TFII could bind to the regulatory elements of *Pax6* and *Otx2* genes, and directly regulate their expression (Fig. 1). Most likely, the regulation is conserved in both mouse and human, because several factors essential for RPE development, including *Pax6*, *Otx2* and *Mitf*, display similar expression profiles in the prospective RPE region of the double mutants and in *siCOUP-TFI/TFII*-treated human ARPE19 cells when compared with the controls. Clearly, *COUP-TF* genes specify the RPE cell fate through inhibiting *Pax6* and activating *Otx2* and *Mitf* in the progenitor cells of pRPE [19].

The expression of COUP-TFs is higher at the temporal optic vesicle at E9.5 [19]. Interestingly, it is the temporal RPE that transdifferentiates into a NR-like structure in the double mutant at E11.5 [19]. It is likely that specification of the RPE is regulated not only along the dorso-ventral axis, but also along the naso-temporal axis, resembling that of the neural retina.

4.2.2. Function of COUP-TF genes determines the boundary between NR and vOS—A boundary between the neural retina and optic stalk is established at the optic disc, an exit for the retinal ganglia cell axons from the eye and an entrance for the blood vessels to the eye [122]. *Pax2*, a vOS marker, and *Pax6*, a NR marker, antagonize each other to establish the sharp boundary between the NR and vOS [39, 123]. *Vax1* and *Vax2* genes, encoding two homeobox transcription factors, may cooperate to inhibit the expression of *Pax6*, and then participate in the ventralization of the optic vesicle, including NR-vOS boundary formation [124]. The expression patterns of COUP-TFs at the proximo-ventral optic vesicle strongly suggest that they might play a crucial role in the specification of the

NR and vOS identities. As expected, the NR-vOS boundary shifts proximally in *COUP-TF* double mutants, accompanying the reduced expression of *Vax1/Pax2* in the OS, *Vax2* in the ventral NR, and increased expression of *Pax6* in the NR [19]. These observations suggest that *COUP-TF* genes are essential factors to balance the expression of *Pax6* and *Pax2* during the early development of the optic cup. *COUP-TF* proteins not only directly repress *Pax6* expression in the ventral optic vesicle; but also indirectly inhibit *Pax6* expression through enhancing the expression of *Vax1/2* genes. As expected, the repression of *Pax6* expression is attenuated in the double mutants; thus *Pax6* expression is enhanced and extends into the prospective vOS territory, resulting in a proximal shift of the NR-vOS boundary [19].

4.2.3. Function of *COUP-TF* genes in the dorsal optic stalk—The specification and differentiation of NR, RPE and vOS identities have been well studied. Nonetheless, where the dOS is originated and how the dOS is specified are still not clear. The expression of *COUP-TFI* and *-TFII* is highest at the multi-layered pdOS in the early optic vesicle at E9.5, and remains high at the multi-layered dOS at E10.5. Their expression is markedly decreased in the single-layered dOS at E11.5. *Pax2* is expressed throughout the optic stalk with a ventral high-dorsal low gradient at E10.5. One day later, the expression of *Pax2* in the OS begins to divert along the disto-proximal axis. At E11.5, *Pax2* expression is distributed evenly in both the proximal dOS and vOS, but is specifically reduced at the single-layered region of the distal dOS. Later, the expression of *Pax2* is excluded from the dOS, and can only be detected in the vOS. However, the prospective dOS maintains a multiple-layered structure in the *COUP-TF* double mutants even at E14.5. Furthermore, the expression of *Pax2* remains high at the mutant dOS at E12.5 and E14.5, indicating that the down-regulation of *Pax2* is probably one of the necessary events for the specification and differentiation of dOS lineage [19]. Taken together, *COUP-TF* genes are essential determinants of the dOS identity.

4.2.4. Function of *COUP-TF* gene in the neurogenesis of the neural retina—In the neural retina, the expression of *COUP-TFI* displays a dorsal-low and ventral-high gradient, while the expression of *COUP-TFII* displays an opposite gradient [17, 19, 125], indicating that *COUP-TF* genes might be involved in the appropriate formation of the topography map from retinal ganglion cells to the midbrain. However, no abnormal projection from the retinal ganglion cells to the superior colliculus (SC) of the midbrain is observed in *COUP-TFI* gene null mutants (Tang, Tsai and Tsai, unpublished observations), suggesting that the gradients of *COUP-TF* genes along the dorso-ventral axis of the NR are not related to the development of the retinotopic projection map of retinal ganglion cells. In the wild-type adult murine neural retina, more S-opsin expressing cone photoreceptor cells are localized at the ventral than the dorsal region; whereas M-opsin expressing cone photoreceptor cells generate a reverse pattern along the dorso-ventral axis [17, 126, 127]. In the adult *COUP-TFI* null mouse retina, S-opsin expressing cone photoreceptor cells distribute evenly along the dorso-ventral axis, and the number of M-opsin cone photoreceptors is higher in the ventral retina than in the dorsal retina. In adult eye-specific *COUP-TFII* mutant retina, the number of S-opsin photoreceptors at the dorsal area increases slightly, and the localization of M-opsin photoreceptors is not changed at all [17]. As

expected, overexpression of either COUP-TFI or -TFII causes an increase in the number of cone photoreceptor cells in the retina [125]. The BMP pathway controls the dorsal-ventral patterning of the neural retina [128, 129]. Consistent with the cone photoreceptor defects observed in *COUP-TFI* null mutant, in retina-specific BMPR CKO (*BMPRIa*^{-f/x}; *BMPRIb*^{+/-}; *Six3-Cre*), the number of S-opsin cone photoreceptors increases at the dorsal retina, and the number of M-opsin cone photoreceptors decreases. In addition, the expression of COUP-TFI is enhanced in the retina of BMPR CKO mice at E14.5, whereas the expression of COUP-TFII is completely diminished. Moreover, both COUP-TFI and -TFII strongly repressed luciferase activation of the S-opsin enhancer/promoter induced by Crx and ROR β 2 *in vitro* [17]. All together, *COUP-TF* genes function downstream of BMP signals to participate in the specification and differentiation of S/M-opsin expressing cone photoreceptors along the dorso-ventral axis of the NR.

In the postnatal neural retina, the expression of COUP-TFI is detected in Müller glia, bipolar and ganglion cells, as well as in many subtypes of amacrine cells positive for GABA, calbindin, GlyT1, ChAT, or parvalbumin. The expression of COUP-TFII is mainly examined in GlyT1 labeling glycinergic amacrine cells [17, 125]. Forced expression of either *COUP-TFI* or *-TFII* results in the increase of GlyT1-positive amacrine cells in INL, implicating that *COUP-TF* genes may also participate in the development of amacrine cells [125]. It will be interesting to distinguish the endogenous functions of *COUP-TF* genes in the specification of amacrine cells and other cell types *in vivo* in the future studies.

4.3. CONCLUSION: COUP-TFs are key regulators in the early morphogenesis of the murine eye

In summary, there are four main domains: pdOS, pRPE, pNR and pvOS, along the dorso-distal and ventro-proximal axis of the early optic vesicle in the murine eye. COUP-TFs are expressed highest in the pdOS, highly in the pRPE, and relatively low in the ventral pNR and pvOS (Fig 2A). Furthermore, COUP-TFs are the essential regulators for the morphogenesis of the murine eye. The presence of COUP-TFs in the pdOS and dOS is necessary for the repression of *Pax2* gene expression; such down-regulation is crucial for the differentiation of dOS (Fig 2B). In pRPE and differentiating RPE cells, COUP-TFs ensure the specification of RPE fate by inhibiting the expression of *Pax6* and activating the expression of *Otx2* and *Mitf*. *Pax6* and *Otx2* are the direct downstream targets of COUP-TFs in RPE (Fig 2C). COUP-TFs also play a crucial role to maintain the balance between *Pax6* and *Pax2*, which are important players for the formation of the NR-vOS boundary. COUP-TFs repress the expression of *Pax6* in the NR directly; in addition, they may promote the expression of the *Vax1/2* gene to inhibit *Pax6* indirectly (Fig 2D).

5. COUP-TFs in eye development in other organisms

5.1. Seven-up in the *Drosophila* eye

Seven-up (*svp*) is a homolog of COUP-TFs in *Drosophila*. The eye of *Drosophila* is comprised of photoreceptor, cone and pigment cells. There are eight photoreceptor cells (R cells); R1–R8. The R8 cell is differentiated first, then R2/R5, R3/R4, R1/R6, with R7 last [130–132]. R1, R3, R4 and R6 cells are transformed to R7 cells in the absence of *svp*,

indicating *svp* is necessary for the appropriate differentiation of R1, R3, R4 and R6 cells [52, 133]. Recent studies reveal that *seven-up* is necessary and sufficient to program the differentiation of R1/R6/R7 precursors [134].

5.2. *svp*[40], *svp*[44] and *svp*[46] are expressed in the developing eye of zebrafish

To identify the *COUP-TF* homologous genes in zebrafish, it was proposed that some characteristics of the *Drosophila* *svp* gene in eye and/or embryonic CNS development could be conserved in zebrafish. Three *COUP-TF* homologous genes: *svp*[40], *svp*[44] and *svp*[46] were identified and cloned in zebrafish [53, 54]. *Svp*[40] transcripts are first examined in 11–12 hpf embryos. The expression of *Svp*[40] is detected in the posterior part of the optic vesicle at 14 hpf, and at the center part of the neural retina at 22 hpf. *Svp*[44] is also expressed in the zebrafish eye. The expression of *svp*[44] transcripts is weakly detected at the center part of the neural retina at 20 hpf, and remains there with a relatively higher level at 24 hpf. As early as 11–12 hpf, transcripts of *Svp*[46] are detected throughout the diencephalon and optic vesicle primordia. At 13 hpf and 14 hpf, its expression increases at the posterior edge of the optic vesicle. The expression of *Svp*[46] generates a lateral low-medial high gradient along the optic vesicle between 14 hpf to 15/16 hpf. At 17 hpf, *svp*[46] expression is examined at the medial part of the optic vesicle and is highest in the region of the optic stalk. In 22 hpf embryos, the *svp*[46] gene is mainly detected in the neural retina and optic stalk [53, 54]. All three *svp* genes of zebrafish are expressed in the eye.

5.3. *xCOUP-TFA* and *-TFB* are expressed in the developing eye of *Xenopus*

There are three *COUP-TFs*; *xCOUP-TFA*, *-TFB* and *-TFC* in *Xenopus* [47–49], and the expression of *xCOUP-TFA* and *-TFB* was examined [48]. At 18 hpf, *xCOUP-TFA* is expressed in three brain areas: the prospective mid/hind brain region, anterior-most parts of the neural fold, and the eye anlagen. At tailbud stages, *xCOUP-TFA* is detected in brain regions in and around the eye, in the developing branchial arches, and in the somites. *xCOUP-TFB* is strongly expressed in the eye anlagen at 18 hpf, and in the dorsal eye at 25 hpf [48].

6. Human *COUP-TFs* and ocular diseases

The human *COUP-TFI* gene is localized to chromosome 5 at 5q14. Several individuals with deletion on the chromosome 15q region, including the *COUP-TFI* gene, were first documented in two independent studies in 2009. Cardoso et al. reported three patients with 5q14.3-q15 deletions, exhibiting peri-ventricular heterotopia, mental retardation, and epilepsy. Coloboma and left eye exotropia are observed in a 7-year-old boy [135]. Brown et al. identified *COUP-TFI* deletion in a 4-year-old girl with profound deafness, a history of feeding difficulties, dysmorphism, strabismus and developmental delay [136]. In 2013, an eight-year-old boy displays symptoms of optic atrophy, dysmorphism and global developmental delay. MRI images reveal the small optic nerves and optic chiasm in the patient. The deletion of 5q15 is confirmed, and *COUP-TFI* is localized in this deleted region and is the strongest candidate gene for the phenotypes observed [137]. One of our latest studies discovers six patients with either point mutations or deletion mutations of the *COUP-TFI* gene [138]. Patients show cerebral visual impairment (CVI) and/or optic nerve

abnormalities such as pale optic disc, and small and large excavation. Point mutations of *COUP-TFI* were identified from patient 1 (c.344G>C, p.Arg115Pro), patient 2 (c.339C>A, p.Ser113Arg), patient 3 (c.755T>C, p.Leu252Pro) and patient 6 (c.335G>A, p.Arg112Lys). Three mutations (p.Arg112Lys, p.Ser113Arg and p.Arg115Pro) are in the DBD domain of COUP-TFI, and one mutation (p.Leu252Pro) is in the LBD domain. Patient 2 has a small optic nerve and chiasm by MRI images. Patient 6 is a 35-year-old female with autism spectrum disorder and marked obsessive-compulsive behaviors. *In vitro* luciferase assays demonstrate that all four mutated proteins lost their capability to fully activate the COUP-TFI reporter [138]. Most likely, *COUP-TFI* haploinsufficiency in humans lead to ocular abnormalities.

Human *COUP-TFII* is localized at 15q26 of chromosome 15. Deletion of a region on chromosome 15q26.1-26.2, where the *COUP-TFII* gene resides, is highly associated with life-threatening Bochdalek-type congenital diaphragmatic hernia (CDH) [139, 140]. *COUP-TFII* conditional null mutants with Nkx3-2Cre display CDH, indicating that probably *COUP-TFII* is one of the etiological factors causing CDH [38]. However, whether *COUP-TFII* deficiency in humans results in any symptoms of ocular disease still needs to be investigated.

7. Prospective future: COUP-TF genes, eye development, and beyond

The expression and functions of COUP-TFs are conserved to ensure the appropriate differentiation and maturation of the eye from invertebrate to mammalian. In addition, the genetic networks regulated by COUP-TFs in the RPE seem also evolutionarily conserved, since a few genes, which are mis-modulated in the prospective RPE of the *COUP-TFI/TFII* double mutant mice, show similar regulatory profiles in the *siCOUP-TFI/TFII*-treated human ARPE19 cells [19]. Moreover, the double knockout mutant embryos generate severe phenotypes of coloboma, a human congenital ocular disease accounting for 3.2–11.2% of blind children worldwide [141], and compromised the dOS and vOS [19]. Consistently, eight of eleven patients reported show various ophthalmological abnormalities, such as coloboma, eye exotropia, optic atrophy, amblyopia, and strabismus, as well as CVI [135–138]. Furthermore, almost every individual with *COUP-TFI* haploinsufficiency also show developmental delays and intellectual disabilities. Clearly, the investigations on COUP-TFs and the eye will benefit our understanding of the etiology of human ocular diseases and beyond.

Acknowledgments

We thank Ms Jodie Hebert for assistance with the manuscript. This work was supported by National Natural Science Foundation of China (81360124 to K.T.), National Institutes of Health Grants R01 HL076448 (to S.Y.T.), R01 DK45641 (to M.-J.T.), HL114539, P01 DK059820 and U19 DK062434 (to M.-J.T. and S.Y.T.), and core facilities of the Diabetes and Endocrinology Research Center (DK079638) and Duncan Cancer Center.

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Highlights

COUP-TFs are highly expressed in the optic vesicle and optic cup.

COUP-TFs are essential regulators for the development of early murine eye.

COUP-TFs can either activate or inhibit gene expression

COUP-TFI mutant leads to human ocular abnormalities.

Fig. 1.

Molecular mechanisms of COUP-TF action. (top panel) COUP-TFs repress gene expression by directly binding to the DR (direct repeat) site of the gene, such as *Pax6* in the eye [19], etc. (Table 1). (Bottom panel) COUP-TFs activate gene expression through forming a modulatory complex with the Sp1 transcription factor, which binds at the *Sp1 site* of the gene, such as *Otx2* in the eye [19], etc. (Table 2).

Fig. 2.

COUP-TF genes and eye development. (A) The expression of COUP-TFs and other key regulators in four domains of the early optic vesicle. Along the dorso-distal and ventro-proximal axis of the early optic vesicle, COUP-TFs are expressed highest in the pdOS, highly in the pRPE, and relatively low in the ventral pNR and pvOS. (B) COUP-TFs are necessary for the differentiation of dOS. (C) COUP-TFs are crucial determinants for the RPE identity. (D) COUP-TFs and the formation of the NR-vOS boundary. dOS, dorsal optic stalk; NR, neural retina; pdOS, presumptive dorsal optic stalk; pNR, presumptive neural retina; pRPE, presumptive retinal pigmented epithelium; pvOS, presumptive ventral optic stalk; vOS, ventral optica stalk.

Table 1

List of genes repressed by COUP-TFs directly through DR binding site

Gene	Tissue or Cell Type Studied	References
Pax6	Eye	[19]
Hey2	Heart (Atrium)	[27]
	HUVEC cell	[71]
Irx4	Heart (Atrium)	[27]
Mlc2v	Heart (Atrium)	[27]
FoxC1	HUVEC cell	[71]
Np-1	HUVEC cell	[71]
VEGFR1	Vascular system	[72]
Wnt10b	Mesenchymal cell	[73]
ApoCIII	HepG2	[74–76]
ApoAI	HepG2	[74–76]
Hemopexin	HepG2	[77]
PPAR- α	HepG2	[78]
CYP2D6	HepG2	[79]
CYP17	Sertoli	[80]
ApoVLDLII	Liver	[81, 82]
Lactoferrin	Uterus	[83, 84]
Oxytocinin	P19 EC cell	[85, 86]
Oct4	P19 EC cell	[87, 88]
	Human ESC	[89]
PGC1 α	White adipocyte	[28]
PPAR- γ	3T3-L1 cell	[30]
Insulin II	HeLa cell	[90, 91]
Dax1	JEG-3 cell	[92]
spActin (CyIIIb)	Sea urchin	[55]
CYP3A		[93]
Preproenkephalin A		[94]
Arrestin		[95]
MMTV		[70]

Table 2

List of genes activated by COUP-TFs directly through Sp1 site

Gene	Tissue or Cell Type Studied	References
Otx2	Eye	[19]
IGF1	Cerebellum	[16]
NRP1	Telencephalon	[20]
NRP2	Telencephalon	[20]
	Lymphatic system	[24]
Tbx5	Heart (Atrium)	[27]
Ang1	Vascular system	[101]
E2f1	HUVEC cell	[71]
Eya1	Kidney	[36]
WT1	Kidney	[36]
Sox9	Mesenchymal cell	[73]
HIV-1	Microglial cell	[102]
NGFI-A	Mesenchymal cell	[100]