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Regulatory potential of COUP-TFs in development: stem/progenitor cells

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Abstract

The formation of complex organisms is highly dependent on the differentiation of specialized mature cells from common stem/progenitor cells. The orphan nuclear receptors chicken ovalbumin upstream promoter transcription factors (COUP-TFs) are broadly, but not ubiquitously, expressed in multiple tissues throughout embryonic development and COUP-TFs are indispensable for proper organogenesis. Recently, growing evidence suggests a critical role of COUP-TFs in multiple aspects of stem/progenitor cell biology. In this review, we highlight the progress of COUP-TFs function and its underlying mechanism in driving stem/progenitor cell self-renewal, lineage specification, differentiation, maintenance, and cell identity in diverse tissue types. These studies provide novel insights into future clinical utilities of COUP-TFs in stem cell based therapies and in the management of diseases.

Keywords

COUP-TFs; stem/progenitor cell

Introduction

Stem cells are diverse toti-, pluri-, and multi-potent ancestor cells with two remarkable features, the capacity to give rise to functional specialized differentiated cell types (multipotency), and the potential to replenish the stem cell pool (self-renewal). Stem cells can be classified into two major types, pluripotent embryonic stem cells for embryonic development and organogenesis, and multipotent adult/somatic stem cells for tissue homeostasis and regeneration. Postnatal tissue of mammals contains numerous lineage-specific precursors, which reside in specific niches and possess the potential to regenerate mature cell types after injury. Stem/progenitor cell development is tightly controlled and a precise balance is maintained among different cellular events such as self-renewal, lineage commitment, differentiation and apoptosis. Disruption of this balance causes uncontrolled cell growth or cell death, thereby leading to various diseases such as cancer or organ defects.

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Nuclear receptors (NRs) encompass 48 members in human and 49 in mice. Numerous studies demonstrate, either directly or indirectly, a role for members of the NR family in regulating cellular functions of embryonic stem (ES) cells and tissue-specific precursors [1]. More than two decades ago, two distinct but related nuclear receptor members have been discovered in humans: COUP-TFI/EAR-3 [2,3] and COUP-TFII/ARP-1 [4–6]. They were classified into the NR2F subgroup and were named chicken ovalbumin upstream promoter transcription factors (COUP-TFs) [2,3]. Specific ligands have not yet been identified for the COUP-TFs, defining them as orphan members of the NR family. The high degree of sequence homology of *COUP-TFs* among different species makes them the most conserved subfamily of nuclear receptors, and suggests a conserved and vital role of *COUP-TFs* during evolution. Biochemical characterization showed that COUP-TFs function as dimers and bind to a spectrum of imperfect AGGTCA direct or inverted repeats spaced by variable nucleotides [7–9]. *COUP-TFs* were first defined as repressors of the transcription of their target genes [8]. However, emerging evidence suggests *COUP-TFs* also activate a growing list of gene promoters both in vitro and in vivo [7,10].

As the first step towards understanding the biological actions of *COUP-TFs* components, the distribution of COUP-TFs protein has been documented in the mouse, *Drosophila*, zebrafish, frog, and *C. elegans* [7,11,12]. COUP-TFI and COUP-TFII exhibit a partially overlapping yet distinct profile in the early mouse embryo, with high levels of COUP-TFII in the mesenchyme of developing organs whereby COUP-TFI expression is relatively confined to the central nervous system (CNS) [12–14]. Organs that developed by epithelial proliferation and differentiation express COUP-TFI at a considerable level in the epithelial cell; for example, neural stem cells in the proliferation zone of the forebrain and hindbrain, and the neural retina (Figure 1A). Conversely, COUP-TFII is constitutively detected in the undifferentiated mesenchymal precursors (Figure 1B,1C), being switched off in the fully differentiated epithelium of most structures like the kidney, stomach, limb bud etc [12,14]. In accordance with its embryonic distribution, mutations of *COUP-TFI* and *COUP-TFII* in mice result in the malformation of the CNS and mesoderm-derived organs, respectively [13,15–18]. Similarly, COUP-TFII activities are frequently found in the stromal/mesenchymal compartment in a wide range of human tissues, including kidney, stomach, intestine, uterus etc. [19].

The complex spatiotemporal distributions of COUP-TFs offer several clues as to its physiological significance in development and disease. Particularly, the high level of COUP-TFs in stem/precursor cells but not mature cell types is highly suggestive of its biological functions in stem/precursor cell development. The following sections review our current state of knowledge of the molecular features of COUP-TFs in various tissues with a particular emphasis on stem/progenitor cells.

COUP-TFs in embryonic stem cells

Pluripotency is a transient state where a cell has the potential to give rise to all somatic cell types. A set of transcriptional factors comprised of OCT4, NANOG, and SOX2 has been proposed to orchestrate the multipotentiality and stemness in ES cells and induced pluripotent stem cells (iPSC). Over past decades it has become clear that COUP-TFs are part of the regulatory circuitry maintaining stem cell function. Pluripotent P19 embryonic carcinoma cells can develop into all three embryonic germ layers closely resembling those normally found in the embryo, and is widely accepted as a model system for early embryogenesis [20]. Several studies reported that COUP-TFs are activated during retinoic acid (RA) induced differentiation of P19 cells and COUP-TFs bind to the OCT4 promoter to silence its transcript upon RA treatment [21–23]. Additional work indicated that endogenous COUP-TFI is required for proper axonal growth and neuron migration in differentiating P19

cells [24]. Considering the essential role of OCT4 in ES cell development, it is thereby conceivable that COUP-TFs may exert a cell-autonomous influence on stem cell pluripotency and cell fate determination.

During hESC differentiation, expression of two pluripotency factors, OCT4 and microRNAs (miR)-302, were gradually diminished with the concomitant induction of COUP-TFII protein [25,26]. In line with this reciprocal expression profile, OCT4 and miR-302 collaborate to block COUP-TFII expression in the pluripotent state, and OCT4 is itself a target of COUP-TFII during differentiation [25]. Phenotype analyses identify a role of COUP-TFII in activating neural gene expression in early development [25]. Another report verifies the feedback loop among OCT4, miR302 and COUP-TFII, and discovers a link between COUP-TFII and pluripotency as higher reprogramming efficiencies are achieved with COUP-TFII knockdown when mesenchymal cells are being converted into iPSC [26]. The molecular circuit of COUP-TFs is further complicated by epigenetic changes as polycomb repressive complex 2 (PRC2) attenuates RA-mediated activation of COUP-TFs in ES cells [27]. Moreover, sustained expression of COUP-TFI promotes NANOG and extraembryonic endoderm gene expression in murine stem cells [28]. Overall, COUP-TFs exert profound effects in regulating key pluripotency factors and likely are integral components of the transcriptional network controlling stem cell property (Figure 2). Nevertheless, what remain to be determined are the *in vivo* studies on COUP-TFs roles in mediating stem cell development.

COUP-TFs in mesenchymal stem cells

Mesenchymal stem cells (MSCs) are heterogeneous progenitor cells with self-renewal and multipotent differentiation potential. Historically, MSCs were first identified within the bone marrow compartment by Friedenstein as adherent “mechanocytes” or stromal fibroblasts [29]. Soon after its discovery, mesenchymal cells were defined by their capacities to undergo adipocyte, osteoblast and chondrocyte differentiation when exposed to appropriate stimuli. Owing to the mesodermal origin of these lineages, Caplan named those newly isolated precursors as “mesenchymal stem cells” and has since gained widespread acceptance [30].

3.1. COUP-TFs controls white fat and bone conversion

The differentiation of MSCs into different lineages is established and maintained by complex transcriptional networks. The signals that promote one specific path often repress an alternative program. One of the most studied examples is the competition between adipogenesis and osteoblastogenesis in which the Wnt cascade favors osteoblast differentiation while preventing adipocyte development [31,32]. Given the mesenchymal specific expression of COUP-TFII and the reported function of *COUP-TFII* in adipogenesis [33], we wonder whether *COUP-TFII* is required for the commitment of mesenchymal progenitors or for the differentiation of specific mesoderm cell types. The mesenchymal cells lacking COUP-TFII protein preferentially develop into osteoblasts rather than adipocytes, regardless of the culture conditions [16]. This lineage conversion is mirrored by the *in vivo* findings that COUP-TFII haploinsufficiency mice display higher bone density coupled with reduced adiposity in the white fat [16,33]. Consistently, *seven-up (svp)*, a COUP-TF counterpart in *Drosophila*, is required for proper fat cell differentiation [34]. *COUP-TFI* may also participate in bone formation as *COUP-TFI* null mice show a premature fusion of the left and/or right exoccipital bone with the basioccipital bone [7]. Having this phenomenon and the known functional redundancy between these two receptors [17], leaves open the possibility of whether *COUP-TFI* itself conducts a role in fat and bone specification.

3.2. COUP-TFII regulates muscle and brown fat identity

Adipocytes share a common mesodermal origin with skeletal muscle, and an inverse correlation between muscle and fat mass has been proposed. For instance, myostatin null mice show a widespread increase in skeletal muscle, whereas the fat tissue is greatly reduced [35]. Significantly, *COUP-TFII* heterozygous mice also display enhanced muscle mass with suppression of fat formation [33], which prompts us to further examine the regulatory property of *COUP-TFII* in myoblast and adipocyte lineage specification. Matching previous reported results [36,37], a marked increase in myoblast differentiation was found in *COUP-TFII*-ablated precursors in comparison to wild type cells [16].

Two different fat tissues exist in mammals: white adipose tissue (WAT) is specialized to store energy, while brown adipose tissue (BAT) burns fat to produce heat. There are two types of BAT with distinct developmental origins. Classical brown fat was derived from a progenitor expressing myoblast marker *Myf5* [38]. Different “brown-like” cells within WAT do not come from the *Myf5* lineage and have been called “beige” cells [39]. Unexpectedly, the white fat in *COUP-TFII* heterozygous mice display a “browning” effect and likely contain “beige” adipocytes with many of the features of BAT, including enhanced mitochondrial biogenesis, elevated mitochondrial volume density, and increased levels of brown fat markers [33]. As a consequence of this beige fat development, mutant mice have increased energy expenditure and are resistant to high-fat diet induced obesity [33]. Intriguingly, prior studies have documented that COUP-TFs modulate transcription of various genes involved in fat metabolism including apolipoprotein for fatty acid transport, enzymes for β -oxidation and fatty acid synthesis [10]. Thereby, it will be interesting to further investigate the functional relevance of *COUP-TFs* in BAT development, in particular the differentiation of two BATs from their distinct precursors.

3.3. COUP-TFII directs lineage decision of the mesenchymal cell

Osteoblasts and chondrocytes are two major cell types in the vertebrate skeletal system, arisen from the common progenitors. Genetic pathways promoting either chondrocyte or osteoblast differentiation are mutually exclusive. Inactivation of COUP-TFII in MSCs not only results in a bidirectional cell fate change between adipocytes and osteoblasts, but also leads to a loss of chondrogenic program as COUP-TFII-deficient progenitors do not undergo appropriate differentiation into chondrocytes [16]. Consistently, COUP-TFII mutants exhibit severe defects in cartilage formation with delayed chondrocyte hypertrophy in the growth plate [16].

Following 5-azacytidine stimulation, mesenchymal cells concurrently develop into multiple mesoderm cell types with morphological and biochemical features of the myoblast, adipocyte, osteoblast, and chondrocyte [40]. Additional evidence for a regulatory role of COUP-TFII in driving mesenchymal cell plasticity comes from experiments showing that loss of COUP-TFII selectively impedes precursors entering adipogenic and chondrogenic lineages while simultaneously activating the switch to osteoblast and myoblast identity [16]. The molecular basis by which *COUP-TFII* directs the lineage conversion is, at least in part, attributed to the modulation of tissue-specific factors (*Runx2*, *PPAR γ* and *Sox9*) and canonical Wnt cascade [16,41]. Building on these studies, we propose a model to summarize the molecular functions of COUP-TFs in mesoderm tissue development (Figure 3).

COUP-TFs in neural stem/progenitor cells

The vertebrate central nervous system originates from the embryonic dorsal ectoderm. Several crucial events occur during CNS development, including neural induction, regionalization, neurogenesis, migration and neuronal circuit formation, which are all highly

related to the different statuses of neural stem/progenitor cells (NSPCs). COUP-TF proteins are universally detected in neural stem cells, early optic vesicles, developing brain tissues in the mouse [13,17,42–44]. The studies over past decades strongly support the notion that COUP-TFs are master regulators dictating the development of NSPCs at various CNS subareas, including the eye and telencephalon.

4.1. COUP-TFs specify cell fate during eye development

Being a highly specialized structure derived from the ventral forebrain, the eye has been widely used as a model system to investigate the specification and differentiation of neural stem cells. In the absence of *COUP-TFI/-TFII* proteins, progenitors in the retinal pigmented epithelium adopt a neural retinal fate, and the stem cell pool in the neural retina expands at the expense of the precursors in the ventral optical stalk, while the progenitors in the dorsal optic stalk gain ventral optic stalk identity [17]. Moreover, COUP-TFI and COUP-TFII proteins are present in dorsal or ventral retinal neural stem cells, respectively, and both genes play cell-autonomous functions in the neurogenesis of the neural retina [17,45]. The effects of COUP-TFs in the eye development seem highly conserved, considering that *seven-up* is absolutely required for specifying photoreceptor subtypes during *Drosophila* compound eye development [46]. This finding is substantiated by the gain-of-function studies in which ubiquitous expression of the *svp* protein causes transformation and cell fate changes of photoreceptors depending on the developmental stages when *svp* is activated [47]. Additional works further demonstrate *svp* is necessary and sufficient to direct the binary fate choice of photoreceptor precursors [48]. Likewise, two zebrafish cognates of COUP-TFs, *svp*[44] and *svp*[46], are regionally distributed within the eye rudiments, suggesting a possible involvement in eye patterning and/or cell differentiation [49]. Together, our studies and others firmly established an irreplaceable function of COUP-TFs in determining stem cell identities in eye morphogenesis.

4.2. COUP-TFs and telencephalon development

The development of neural stem cells in the telencephalon is probably one of the most complicated events during embryogenesis. Emerging evidence strongly suggests that COUP-TFs are key players orchestrating the maturation of telencephalon NSPCs. First, COUP-TFs are intrinsic factors defining the competence of NSPCs. Both in vitro and in vivo data indicate that COUP-TF genes likely modulate the gliogenic competence and neuropotency of NSPCs in the early CNS development [43]. Second, COUP-TF factors, especially the *COUP-TFI* gene, define the spatial specification of neural precursor cells as COUP-TFI regulates the early neocortical regionalization [50]. Further analyses using the dorsal telencephalon specific *COUP-TFI* knockout indicates COUP-TFI is necessary for the cortical patterning between frontal/motor and sensory areas [51]. Additional loss- and gain-of-function studies reveal that *COUP-TFI* promotes ventral cortical identity [52]. Third, COUP-TFs are indispensable for the genetic control of diverse cell fates during NSPCs development, evidenced by the loss of both layer IV neurons and subplate neurons in COUP-TFI mutant animals [53]. In *Emx1*Cre mediated cortex-specific *COUP-TFI* inactivation mice, the corticospinal motor neurons in the somatosensory cortex do not develop properly [54]. Another mutant mouse, having *COUP-TFI* depleted in its subventricular precursors and postmitotic cells, shows a decrease in late-born, caudal ganglionic eminence (CGE)-derived cortical interneurons with the concurrent increase of early-born cortical interneurons originated from medial ganglionic eminence (MGE) [44]. Moreover, COUP-TFII also modulates the proliferation and survival of granule cells in the mouse cerebellum [55]. Fourth, COUP-TFs factors have been linked to cell migration, which is required for the maturation of NSPCs, in the embryonic mouse brain. At the ventral forebrain, *COUP-TFI* and *COUP-TFII* genes control the tangential migration [56]. The latest study from our laboratory demonstrates that the *COUP-TFII* gene drives the migration

of CGE-derived Pax6⁺ cells into the basomedial amygdala nucleus [42]. Finally, both *COUP-TFI* and *COUP-TFII* genes influence the terminal differentiation of NSPCs by affecting axon guidance, innervation and arborization in the nervous system. In the absence of *COUP-TFI*, the abnormal axon guidance and arborization are found in several regions of the periphery nervous system [57]. In addition, guidance and innervation defects are presented by aberrant thalamocortical projections [53] and the corpus callosum projections, as well as the failure of hippocampal commissure and anterior commissure to cross the midline [58] in mutant animals. The involvement of COUP-TFs in NSPC maturation is functionally conserved in other species. *UNC-55*, the COUP-TF ortholog in *C. elegans*, is expressed in the late-born ventral D class GABAergic (VD) motor neurons but not in early-born dorsal D (DD) neurons [59]. VD neurons lacking UNC-55 protein gain the synaptic pattern of the DDs, whereby ectopic activation of UNC-55 in DD neurons block dorsal synaptogenesis and render them to adopt late-born ventral features [59–61]. Molecularly, *UNC-55* represses *hbl-1* and *irx-1* transcripts in the VDs to dictate synaptic remodeling in *C. elegans* [62,63]. Like *UNC-55*, *Drosophila seven-up* represses *hunchback* expression in neural stem cells to allow neuroblast daughters to gain late-born cell fates [64,65]. Without a doubt, COUP-TF factors are master regulators controlling multiple facets of NSPCs to produce functional neuronal networks.

COUP-TFs in kidney precursor cells

The mammalian kidney arises from the intermediate mesoderm. The reciprocal tissue interplay between the metanephric mesenchyme and the ureteric bud drives the process of kidney formation, while Gdnf-Ret cross talk controls nephron synthesis [66]. The induction of kidney mesenchyme is mediated by the coordinate interactions within *Osr1/Eya1/Pax2/Six2* hierarchy [66]. COUP-TFII has an early onset of expression in kidney precursors and ablation of *COUP-TFII* results in the absence of metanephric mesenchyme and the misexpression of key nephrogenic factors, including *Eya1*, *Pax2*, *Six2* and *Gdnf* [18]. Thus, *COUP-TFII* acts in parallel with *Osr1*, the earliest gene that marks the kidney progenitors, to control *Eya1* transcription, thereby activating other downstream effectors and initiating metanephric lineage specification and ureteric bud outgrowth (Figure 4). Additionally, COUP-TFII-deficient mesenchymal cells exhibited decreased proliferation coupled with increased apoptosis, suggesting a function of COUP-TFII in the maintenance of the metanephric mesenchyme [18]. Accordingly, in response to mitogenic EGF signaling and its secreted ligand, *Spi*, *seven-up* is activated, leading to the expression of cell cycle regulators *string* and *cyclinE*, thus stimulating cell proliferation during Malpighian tubule growth [67,68]. Taken together, these data reveal a crucial function of *COUP-TFs* in regulating early patterning in kidney organogenesis.

COUP-TFs in vascular cells

The formation of a mature vertebrate vascular system consists of two major steps; vasculogenesis generates a primitive vascular network from endothelial precursor cells, and angiogenesis remodels the primitive plexus into organized vascular networks including arteries, veins and capillaries. COUP-TFII transcripts are found in the visceral mesoderm and the sinus venosus and *COUP-TFII* loss leads to embryonic lethality with angiogenic defect as the mutant primitive plexus fails to develop into large and small microcapillaries [69]. The compromised angiogenesis is mainly contributed by the direct regulation of *Ang1* in the surrounding mesenchymal tissues, and *VEGFR1* and *E2F1* in the endothelial cell compartment of *COUP-TFII* mutants [69–71].

Endothelial cells (EC) form a continuous lining inside the veins, arteries and lymphatic vessels and the EC identity was either genetically predetermined or acquired through

external environmental stimuli. The general concept regarding arterial specification is that *Shh* lies upstream of *VEGF* and *Notch* cascade to stimulate arterial specific marker ephrin B2, and failure to activate *Notch* signaling gives rise to veins by default [12,72]. COUP-TFII is detected in the venous ECs, but not the arterial counterparts [73]. Endothelium-specific disruption of *COUP-TFII* converts the features of the veins into arterial-like as shown by the ectopically expressed arterial markers in mutant venous ECs [73]. More importantly, the COUP-TFII-deficient venous ECs activate hematopoietic cell clusters and recruit smooth muscle cells, two primary functional characteristics of arteries [73]. Mechanistically, COUP-TFII confers vein identity through inhibition of the *Notch* signaling pathway in venous ECs, thereby interfering with arterial phenotypes [71,73]. The effect of COUP-TFII in EC fate decision is evolutionary conserved as COUP-TFII orthologues also promote venous commitment in zebrafish and *Xenopus laevis* [74].

Prospective remarks and future direction

In this review, we summarize recent progress of studies on COUP-TFs in the stem/progenitor cell field across various cellular processes. Matching its embryonic distribution, malfunction of *COUP-TF* genes leads to abnormal organogenesis and neurogenesis, and aberrant levels of COUP-TFs protein are linked to congenital diseases such as congenital diaphragmatic hernia and congenital ocular coloboma [17,75]. Contrary to its critical functions in embryonic development, COUP-TFs are dispensable for the maintenance of developed tissues because depletion of COUP-TFII in adult organs does not generate discernible abnormalities. Having the critical effects of COUP-TFs on stem/progenitors cells, one would expect that COUP-TFs may exert a function under certain pathological conditions such as injury, in which progenitor cells activate, expand, and differentiate to repair damaged tissue. This is indeed the case as ongoing studies in the laboratory indicate COUP-TFII plays critical roles in stem cell-mediated regenerative myogenesis. Moreover, COUP-TFs are involved in various human diseases, such as cancer, metabolic syndrome, and renal diseases [18,33,76]. Collectively, these works point out a potential for COUP-TFs as drug intervention targets and provide an impetus to identify small molecules that inhibit or activate COUP-TFs' activities. A recent report identifies a ligand-binding pocket in COUP-TFII, and COUP-TFII is a low-affinity RA-responsive receptor [77], which provides an example to isolate compounds that selectively modulate COUP-TF activities and lay the foundation for using COUP-TF antagonists/agonists as new therapeutic agents for disease control.

Given the diverse activities of COUP-TFs in development, a genomic view of COUP-TF-dependent regulatory networks with tissue specificity will shed more light on the underlying basis of COUP-TF's control. DNA-binding proteins like COUP-TFs bind preferentially to certain DNA sequences to convey its action. Thus, the genome-wide mapping of COUP-TF's binding motifs through ChIP-seq will complement the microarray studies and reveal the global targets impacted by COUP-TFs. The physical interplay of COUP-TFs with lineage-affiliated transcriptional factors and signaling cascades through protein-protein interaction brings another level of complexity to the regulation of these receptors [16,41,76]. These studies lead us to search for additional binding proteins that further specify the actions of COUP-TFs. To this end, a systematic proteomic approach such as immunoprecipitation followed by mass spectrometry will be particularly informative in defining partners associated with COUP-TF receptors.

During development, stem/progenitor cells convert into diverse cell lineages and each mature cell type is responsible for specialized cellular functions for living organisms. Therefore, accurate knowledge of the cellular circuitry underlying stem/progenitor cell development is essential for exploiting the power of these cells in clinical usage. Here, we

present evidence to establish the critical functions of COUP-TF receptors in the developmental regulation of stem/progenitor cell property and propose a framework through which these observations can be translated into therapeutic applications using COUP-TF antagonists/agonists in disease treatment.

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Highlights

1. COUP-TFs are highly expressed in progenitor/stem cells.
2. COUP-TFs are important for the arterial/venous fate determination.
3. COUP-TFs are important for the mesenchymal cell fate determination.
4. COUP-TFs are important for the formation of the metanephric mesenchyme.
5. COUP-TFs are important for the neuronal fate determination.

Fig. 1. The expression profiles of COUP-TFs in the early embryonic development. The immunohistochemical assay with COUP-TF antibodies were performed on E10.5 mice embryos. COUP-TFI protein is detected in the proliferating zone of the forebrain and hindbrain, and in the neural retina, where NSPCs are localized (A). COUP-TFII is highly expressed in the mesenchymal compartment of developing organs, such as the surrounding mesenchyme of the foregut, lung bud and the septum transversum (B); a higher magnification of the boxed region is shown in C.

Fig. 2.

Diagram illustrates the molecular networks of COUP-TFs interactions with pluripotency factors. COUP-TFII is inhibited transcriptionally by OCT4 and post-transcriptionally by miR302, and COUP-TFII in turn blocks OCT4 expression. PRC2 negatively regulates RA-induced expression of COUP-TFs in pluripotent cells. COUP-TFs play regulatory roles in the maintenance of pluripotency as well as proper induction of endodermal and neural gene expression during differentiation. Pointed arrows indicate activation, blunted arrows represent inhibition.

Fig. 3.

Working model of COUP-TFII as key factor controlling the mesoderm-derived tissue formation. Mesenchymal cells differentiate into white adipocyte, bone, cartilage, muscle and brown fat tissues in response to specific stimuli. COUP-TFII blocks progenitors from entering bone, muscle and brown fat lineages while promoting the development of cartilage and white adipocytes.

Fig. 4. COUP-TFII defines the metanephric mesenchymal fate of kidney precursor cells. COUP-TFII acts in parallel with Osr1 to activate Eya1 and its downstream targets, thereby governing metanephric mesenchyme formation. COUP-TFII also regulates Wt1 expression to control kidney precursor cell survival.