Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4451–4455, May 1994
Developmental Biology

Spatiotemporal expression patterns of chicken ovalbumin upstream promoter–transcription factors in the developing mouse central nervous system: Evidence for a role in segmental patterning of the diencephalon

(steroid receptor/mouse embryo/orphan receptor/brain/development)

YUHONG QIU*, AUSTIN J. COONEY*, SHIGERU KURATANI†, FRANCESCO J. DEMAYO*, SOPHIA Y. TSAI*, AND MING-JER TSAI*‡

*Department of Cell Biology and †V. and M. McLean Department of Biochemistry, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

Communicated by B. W. O'Malley, November 3, 1993 (received for review September 28, 1993)

ABSTRACT **Chicken ovalbumin upstream promoter–transcription factor (COUP-TF) genes encode transcription factors belonging to the orphan subfamily of the steroid/thyroid hormone receptor superfamily. Two COUP-TF counterparts have been cloned from mouse. In an attempt to study the function of these genes in the developing central nervous system (CNS), the spatiotemporal expression patterns of the two mouse genes have been examined by *in situ* hybridization. Both genes are widely expressed in the developing CNS, with patterns that are overlapping yet distinct from each other. The differential expression of murine COUP-TFI and -II in the diencephalon is striking in that high levels of expression from each gene are confined to specific segmental compartments—the neuromeres. Our results suggest that murine COUP-TFs may play important roles in the development and differentiation of the CNS, including the specification of diencephalic neuromeres.**

Chicken ovalbumin upstream promoter–transcription factors (COUP-TFs) belong to the superfamily of steroid/thyroid hormone receptors (1, 2). They are also known as orphan receptors since no ligand has yet been identified. Two members of this group have been cloned from human and are designated COUP-TFI (2), also known as ear-3 (1), and COUP-TFII (3, 4), also named ARP I (5). Like other steroid hormone receptors, each COUP-TF has a DNA binding domain (DBD) and a ligand binding domain (LBD). The amino acid sequences of human (h) COUP-TFI and -II are highly homologous in these functional domains (4). Counterparts of COUP-TFs have been found in many species in the animal kingdom (6–10). These genes all show high homology in their amino acid sequences, indicating a high degree of evolutionary conservation.

Functional characterization has demonstrated that COUP-TFs can repress transactivation of target genes induced by vitamin D_3, thyroid hormone, and retinoic acid receptors (11–14). Repression of the retinoid and thyroid hormone pathways is of special interest since these hormones and their respective cognate receptors are involved in vertebrate morphogenesis (15–18). In the central nervous system (CNS), expression of COUP-TFs has been reported in zebrafish and chicken (7, 8). In chicken embryos, chicken (c) COUP-TFII is expressed transiently in spinal motor neurons and ectopic expression can be induced by a notochord graft (8). This would imply that cCOUP-TFII is functioning as a member of a cascade of transcription factors operating downstream of factors released by the notochord. cCOUP-TFII is also expressed in other parts of the CNS, suggesting that COUP-TFs may function in other regions during neural development. It is therefore important to determine the developmental expression patterns of COUP-TFs in the CNS for each member of this nuclear receptor subfamily. To investigate the *in vivo* functions of COUP-TFs, we have used the mouse model system. Examination of the spatiotemporal expression patterns of COUP-TFs will reveal potential sites of action and will shed light on possible roles of these nuclear receptors in the developing nervous system.

MATERIALS AND METHODS

Cloning of Murine (m) COUP-TF cDNAs. To generate a specific probe to clone the mouse homologs of COUP-TFs, a PCR strategy was used. Two oligonucleotides consisting of sequences from hCOUP-TFI were used as primers (the 5′ primer is from positions 628–647; the 3′ primer is from positions 890–871) to amplify a 270-bp fragment of the LBD of mCOUP-TFs (2). DNA (1 μg) purified from a mouse BALB/c neonatal Uni-ZAP XR cDNA library (Stratagene) was used as the template. An amplified DNA fragment of the correct size was subcloned and sequenced. The sequence confirmed that the DNA fragment encoded part of mCOUP-TFI. It was then used as a probe to screen the same cDNA library. A total of 5×10^5 plaque-forming units were screened. Several positive clones were isolated, subcloned, and sequenced.

Embryo Preparation. Staged mouse embryos were collected and fixed in 4% paraformaldehyde (PFA) overnight at 4°C. They were cryoprotected by sinking in a graded series of sucrose (7%, 15%, and 23% in PBS) and finally embedded in OCT. Serial sections of 10–15 μm were cut using a cryostat, postfixed in PFA, dehydrated, and stored at −20°C.

Probe Preparation. The template cDNAs encoding the whole open reading frames of mCOUP-TFI and -II were subcloned into pBluescript SK+. ^{35}S-labeled antisense and sense RNA probes were synthesized by T3 and T7 polymerase, respectively, according to the manufacturer's conditions (Promega). The probes were degraded to 150–300 bp by limited alkaline hydrolysis. Unincorporated nucleotides were removed by ethanol precipitation.

***In Situ* Hybridization.** *In situ* hybridization was performed as described by Wilkinson *et al.* (19). Briefly, the sections were hybridized at 55°C with RNA probes with a minimum of 10^8 cpm/ml. Washes were performed with high stringency at

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: COUP-TF, chicken ovalbumin upstream promoter–transcription factor; DBD, DNA binding domain; LBD, ligand binding domain; CNS, central nervous system; h, human; m, murine; p.c., postcoitum.

‡To whom reprint requests should be addressed.

65°C. The sections were dipped into Kodak NTB2 emulsion, exposed for 5 days [with the exception of 18.5-day postcoitum (p.c.) embryonic sections, which were exposed for 14 days], developed in Kodak D19 developer, and counterstained with hematoxylin. The sections were photographed with a Zeiss Axiophot microscope.

Although mCOUP-TFI and -II are very homologous at the amino acid level, they share only 80% identity at the DNA level. The differences between the two cDNAs in their open reading frames are evenly scattered over the entire length. The mismatches will be cleaved by RNase digestion and released during the subsequent high temperature wash. From the data (see *Results*), especially sections from 14.5 days p.c., it is clear that some structures are hybridized only to mCOUP-TFI probe, while another structure (oculomotor nucleus) is hybridized strongly only to mCOUP-TFII probe. Therefore, we can state with confidence that the strong hybridization signals are specific, although we do not rule out minor cross hybridization. Sense probes showed background level signals.

RESULTS

Cloning of mCOUP-TF cDNAs. Two COUP-TF cDNAs of 1559 and 1578 bp were isolated from a mouse cDNA library. The two cDNA clones contained complete open reading frames of 435 and 431 amino acids, respectively. Sequence comparisons (Fig. 1) showed that one clone shared 99% identity with hCOUP-TFI at the amino acid level, while the other showed 100% identity with hCOUP-TFII. Therefore, the two cDNAs were designated as mCOUP-TFI and mCOUP-TFII, respectively. Like their human counterparts, the DBDs of mCOUP-TFI and -II were almost identical except for one conserved serine to threonine substitution. The most diverged region between mCOUP-TFI and -II was at the N-terminal domains, which shared only 40% identity.

Expression of mCOUP-TFs. To determine the spatiotemporal distribution of mCOUP-TFI and -II transcripts, *in situ* hybridization was performed on serially sectioned mouse embryos ranging from 7.5 to 18.5 days p.c. Expression of mCOUP-TFI and -II was first observed around 8.5 days p.c., peaked at 14–15 days p.c., and declined before birth (data not shown). Up to 10.5 days p.c., both genes were expressed in a similar pattern in the ventricular layer of the brain (Fig. 2 *A* and *B*). Three expression domains were recognized in the brain at 11.5 days p.c. (Fig. 2 *C* and *D*). The anterior domain included the optic stalk, dorsocaudal portion of the telencephalon, and the thalamus. The medial domain was re-

A

```
mcouptf1 : ATGGCAATGGTAGTTAGCAGCTGGCGAGATCCGCAGGACGACGTGGCCGGGGGCAACCCC
mcouptf2 : --------------C----CG-----C--C--C--------G---C----.........

mcouptf1 : GGCGGCCCCAACCCCGCAGCGCAGGCAGCCCGCGGCGGCGGCGGCGGCGAGCAGCAGCAG
mcouptf2 : ............-T-T---G----C---G--T--CA----C--C-..--T--C-.G--CC

mcouptf1 : GCGGGCTCCGGCGCGCCGCACACGCCGCAGACCCCGGGCCA.....GCCCGGAGCGCCCG
mcouptf2 : --C-C--..-----C----------A-----G--------agggg------CCA--A--

mcouptf1 : CCACCCCCGGCA.CGGCAGGGGACAAGGGCCAGGGCCCGCCCGGTTCAGGC.........
mcouptf2 : --GG---A-A--g----T--C-G-C------...-----TGG---CC-G---agcgacaag

mcouptf1 : ...CAGAGCCAGCAGCACATCGAGTGCGTGGTGTGCGGGGACAAGTCGAGCGGCAAGCAC
mcouptf2 : cag---CAG-----------------------------------------------------

mcouptf1 : TACGGCCAATTCACCTGCGAGGGCTGCAAAAGTTTCTTCAAGAGGAGCGTCCGCAGGAAC
mcouptf2 : --------G-----G----------G--C---------C-C-----G--G------

mcouptf1 : TTAACTTACACATGCCGTGCCAACAGGAACTGTCCCATCGACCAGCACCACCGCAACCAG
mcouptf2 : C-G-GC-----G-----C------C-------------------------------------

mcouptf1 : TGCCAATACTGCCGCCTCAAGAAGTGCCTCAAAGTGGGCATGAGGCGGGAAGCGGTTCAG
mcouptf2 : -----G----------A--------------------A--------T--A---

mcouptf1 : CGAGGAAGAATGCCTCCAACCCAGCCCAATCCAGGCCAGTATGCACTCACAAACGGGGAT
mcouptf2 : A----C--G--------T--------T-CC-AC--G----T---C--G--C--------C

mcouptf1 : CCTCTCAATGGCCACTGCTACCTGTCTGGCTACATTTCTCTGCTGCTGCGCGCAGAGCCC
mcouptf2 : --C-----CT------CG--------C--A--T-----C----------G------

mcouptf1 : TACCCCACGTCGCGTTATGGCAGCCAGTGCATGCAGCCCAACAACATCATGGGCATCGAG
mcouptf2 : --------------C-TC-----T--------------T---------------------

mcouptf1 : AACATCTGCGAGCTGGCAGCCCGCCTCCTCTTCAGCGCCGTCGAGTGGGCCCGCAACATC
mcouptf2 : -----T-----A-----C--A--GA-G----------T----------G------

mcouptf1 : CCGTTCTTCCCGGATCTGCAGATCACGGACCAGGTGTCTCTGCTGCGCCTCACCTGGAGC
mcouptf2 : --C--------T--C--------------------G-C--C--T---------------

mcouptf1 : GAGCTGTTCGTGCTCAACGCGGCCCAGTGCTCCATGCCCCTGCACGTGGCGCCGCTGCTG
mcouptf2 : ------------T-G--T----------------------C--T--C--C-----C--T

mcouptf1 : GCCGCAGCCGGCCTGCACGCCTCGCCCATGTCCGCGGACCGCGTCGTGGCCTTCATGGAC
mcouptf2 : -----T--T----------T--A--------A--C-----G--G--C--T--T------

mcouptf1 : CACATCCGCATCTTTCAGGAACAGGTGGAGAAGCTCAAGGCGCTGCACGTCGACTCTGCC
mcouptf2 : -----A--G-----C--A--G--A----------------A--------------C---

mcouptf1 : GAGTACAGCTGCCTCAAAGCCATCGTGCTATTCACGTCAGATGCTTGTGGCCTGTCGGAT
mcouptf2 : -----T----------G-----A--C--G-----C--------C-----T-----T---

mcouptf1 : GCTGCCCACATCGAAAGCCTGCAGGAGAAATCACAGTGTGCCCTGGAGGAGTATGTGAGA
mcouptf2 : -TA-----TG-G------T-------A--G--C--------TT----A-----C--T--G

mcouptf1 : AGCCAGTACCCCAACCAGCCCAGCCGCTTTGGCAAACTGCTGCTGCGATTGCCCTCTCTT
mcouptf2 : ------------------A-CA--G--C--A--G--CT----T--TC-C--T--C--C

mcouptf1 : CGCACAGTGTCCTCCTCTGTCATCGAGCAACTCTTCTTCGTACGTTTGGTAGGTAAAACT
mcouptf2 : -----G--C--------A-----A------T-G--T-----C----------------C

mcouptf1 : CCCATCGAAACTCTCATCCGAGATATGTTGCTGTCAGGGAGCAGTTTCAACTGGCCTTAC
mcouptf2 : ----------C--------G--------A-----C--C--------T--------A--T

mcouptf1 : ATGTCCATCCAGTGTTCCTAG
mcouptf2 : ---G-A--T--.....A--A
```

B

```
mcouptf2 : MAMVVSTWRDPQDEVPGSQGSQASQAP.....PVPGPPPGAPHTPQTPGQGGP
hcouptf2 : ------------------------.....----------
mcouptf1 : ------S------D-A-GNPGGPNP-Aqaargggggg..e.QQQAGS----------P-A
hcouptf1 : ------S------D-A-GNPGGPNP-AqaargggggageQQQQAGS----------P-A

mcouptf2 : ASTPAQTAAGGQGGPGGPGSDKQQQQQHIECVVCGDKSSGKHYGQFTCEGCKSFFKRSVR
hcouptf2 : ------------------------------------------------------------
mcouptf1 : PA--GTAGDK---P--.....SG-S-----------------------------------
hcouptf1 : PA--GTAGDK---P--.....SG-S-----------------------------------

mcouptf2 : RNLSYTCRANRNCPIDQHHRNQCQYCRLKKCLKVGMRREAVQRGRMPPTQPTHGQFALTN
hcouptf2 : ------------------------------------------------------------
mcouptf1 : ---T--------------------------------------------------NP--Y----
hcouptf1 : ---T--------------------------------------------------NP--Y----

mcouptf2 : GDPLNCHSYLSGYISLLLRAEPYPTSRFGSQCMQPNNIMGIENICELAARMLFSAVEWAR
hcouptf2 : ------------------------------------------------------------
mcouptf1 : -----G-C-------------------Y----------------------L---------
hcouptf1 : -----G-C-------------------Y----------------------L---------

mcouptf2 : NIPFFPDLQITDQVALLRLTWSELFVLNAAQCSMPLHVAPLLAAAGLHASPMSADRVVAF
hcouptf2 : ------------------------------------------------------------
mcouptf1 : --------------S-------------------------------------------------
hcouptf1 : --------------S-------------------------------------------------

mcouptf2 : MDHIRIFQEQVEKLKALHVDSAEYSCLKAIVLFTSDACGLSDVAHVESLQEKSQCALEEY
hcouptf2 : ------------------------------------------------------------
mcouptf1 : ----------------------------------------A--I--------------
hcouptf1 : ----------------------------------------A--I--------------

mcouptf2 : VRSQYPNQPTRFGKLLLRLPSLRTVSSSVIEQLFFVRLVGKTPIETLIRDMLLSGSSFNW
hcouptf2 : ------------------------------------------------------------
mcouptf1 : ---------S--------------------------------------------------
hcouptf1 : ---------S--------------------------------------------------

mcouptf2 : PYMAIQ
hcouptf2 : ------
mcouptf1 : ---S--
hcouptf1 : ---S--
```

FIG. 1. (*A*) Alignment of nucleotide sequences of mCOUP-TFI and -II open reading frames. Start and stop codons are underlined. (*B*) Alignment of amino acid sequences of the entire open reading frames of mCOUP-TFI and -II (mcouptf1, -2) and hCOUP-TFI and -II (hcouptf1, -2). Boxed sequence represents the DBD. LBD includes all the sequences C-terminal to the DBD. Note that there are only 3 amino acid differences between mCOUP-TFI and hCOUP-TFI, while mCOUP-TFII and hCOUP-TFII are identical.

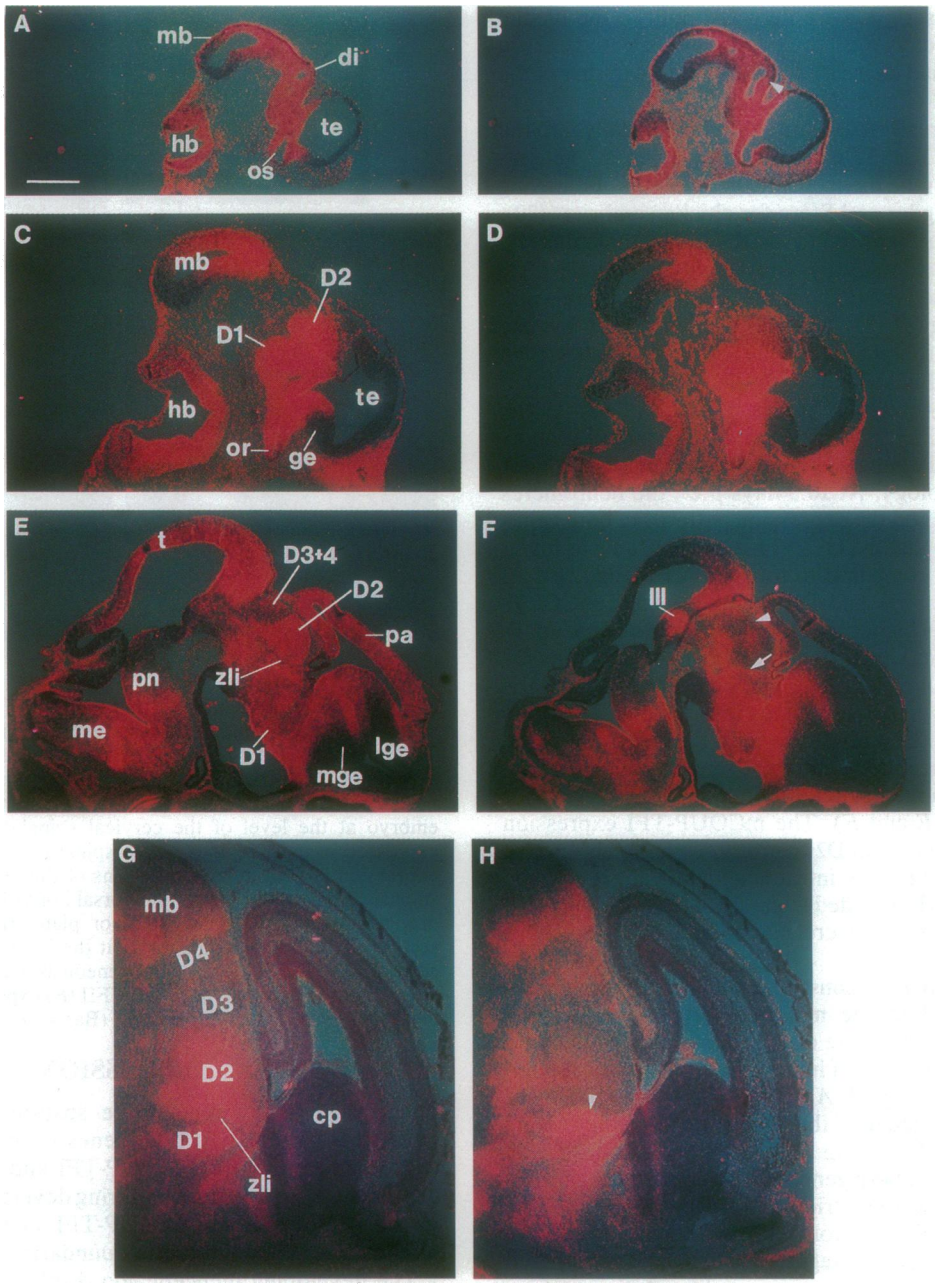

FIG. 2. Comparison of mCOUP-TFI (*A*, *C*, *E*, and *G*) and -II (*B*, *D*, *F*, and *H*) expression patterns in the developing mouse brain by *in situ* hybridization using ^{35}S-labeled RNA probes. Red color represents specific hybridization signals. (*A* and *B*) Parasagittal sections of 10.5-day p.c. embryonic brain. (*A*) Expression of mCOUP-TFI is seen in the optic stalk (os), dorsocaudal part of the telencephalon (te), diencephalon (di), midbrain (mb), and hindbrain (hb) regions. (*B*) Expression pattern for mCOUP-TFII is similar to mCOUP-TFI except for a slightly lower expression in the future D2 region (dorsal to arrowhead) of the diencephalon. (*C* and *D*) Parasagittal section of 11.5-day p.c. embryonic brain. Expression of mCOUP-TFII is weaker in D2 than that of mCOUP-TFI. Note the rostrally restricted expression domain in the midbrain. or, Optic recess; ge, ganglionic eminence. (*E* and *F*) Sagittal sections of 14.5-day p.c. embryo. Expression levels of mCOUP-TFI and -II are high in D1 and the medial ganglionic eminence (mge), and low in D3/D4 regions and the zona limitans intrathalamica (zli). mCOUP-TFI (*E*) is highly expressed in D2, the lateral ganglionic eminence (lge), the pallium (pa), and the tectum (t). In contrast, mCOUP-TFII expression is undetectable in these regions except for the rostral part of the tectum (*F*). Expression of mCOUP-TFII is high in the oculomotor nucleus (III) where mCOUP-TFI expression is minimal. Arrow in *F* points to the D1/D2 boundary and arrowhead indicates the D2/D3 boundary. me, Medulla; pn, pons. (*G* and *H*) Sagittal sections of 18.5-day p.c. forebrain. Expression of COUP-TF genes at this stage is much lower than previous ones since the sections were exposed to emulsion for 14 instead of 5 days. Minimal expression is seen in the telencephalon for mCOUP-TFI and -II. Expression in D1, D3, D4, zli, and rostral midbrain regions is the same for both genes (arrowhead in *H* shows the anterior boundary of zli). Note that expression in D2 is high for mCOUP-TFI and low for mCOUP-TFII. cp, Caudate and putamen. (Bar = 0.5 mm.)

stricted to the future midbrain and the caudal domain started in the middle of rhombomere 1 in the hindbrain and extended caudally. The separation of the anterior and the medial domains was not distinct prior to 11.5 days p.c.

Forebrain. At 10.5 days p.c., the expression patterns of mCOUP-TFI and -II were similar in the future telencephalon (data not shown). Later, the anterior expression domain of mCOUP-TFI extended anteriorly and dorsally in the pallium and, by 13.5 days p.c., the transcripts were detected in most of the pallium (Fig. 2*E* shows 14.5-day p.c. pallium). The mCOUP-TFII expression domain, on the other hand, was restricted more caudally (Fig. 2*F*). At 14.5 days p.c., the lateral and medial ganglionic eminences are well developed. mCOUP-TFI was expressed in both eminences, while the

mCOUP-TFII transcripts were detected only in the medial eminence. By 18.5 days p.c., the expression of both genes in the telencephalon had decreased approximately to that of the background (Fig. 2 *G* and *H*).

The anterior expression domain extended caudally into the diencephalon. mCOUP-TFs were expressed in a segment-restricted fashion in the diencephalic neuromeres. Following the definition by Figdor and Stern for chicken embryos (20), these neuromeres are referred to as D1 (ventral thalamus and hypothalamus), D2 (dorsal thalamus), and D3 and D4 (pretectal region). Based on the mouse embryonic forebrain, Puelles and colleagues (21) have put forth another scheme that divides the forebrain into compartments smaller than those proposed by Figdor and Stern. Our result showed that mCOUP-TFI and -II were expressed differentially in the dorsal thalamus, which is similarly defined by both groups. Henceforth, the following descriptions will employ Figdor and Stern's terminology. At 10.5 days p.c., the neuromeres are not well defined and the expression patterns of both genes were similar except that mCOUP-TFII expression was lower in the future D2 as compared to mCOUP-TFI (Fig. 2*B*). At 11.5 days p.c. when D1 and D2 become visible, mCOUP-TFII expression was higher in D1 than in D2, while mCOUP-TFI was expressed at similarly high levels in both D1 and D2 (Fig. 2 *C* and *D*). At 14.5 days p.c., the D3/D4 region is distinguishable and the zona limitans intrathalamica appears between D1 and D2. Essentially no COUP-TFI or -II expression was detected in this boundary segment and the expression was slightly above background in the D3/D4 region for both genes (Fig. 2 *E* and *F*). The mCOUP-TFI expression domain now extended from D2 rostrally, with the absence of expression in zona limitans intrathalamica (Fig. 2*E*), while that of mCOUP-TFII extended from D1 anteriorly (Fig. 2*F*). Such expression patterns were maintained until 18.5 days p.c. (Fig. 2 *G* and *H*).

Midbrain and Other Regions. The medial expression domain was restricted to the midbrain. At 10.5 days p.c., mCOUP-TFI transcripts were distributed in the tectum with an anteroposterior gradient of intensity with the highest point at the rostral end (Fig. 2 *A*, *C*, and *E*). mCOUP-TFII expression was restricted to the rostral third of the tectum (Fig. 2 *B*, *D*, and *F*). From 16.5 days p.c. onward, the expression domains of both genes narrowed rostrally and, by 18.5 days p.c., they were restricted to an anterior strip of the tectum (Fig. 2 *G* and *H*). From 11.5 to 16.5 days p.c., the oculomotor nucleus in the tegmentum strongly expressed mCOUP-TFII. This nucleus represents the anteriormost somatic motor component along the neuraxis.

mCOUP-TFs were expressed at high levels in a portion of the hindbrain neuroepithelium just lateral to the floor plate at 11.5 days p.c. (Fig. 3 *A* and *B*). This structure colocalizes with the cranial motor nuclei in the hindbrain (22, 23). mCOUP-TFI was also expressed at a lower level in the rest of the hindbrain neuroepithelium while mCOUP-TFII showed background expression (Fig. 3 *A* and *B*). From 10.5 days p.c. onward, both mCOUP-TFs were expressed in spinal motor neurons, the posterior somatic motor components (Fig. 3 *C* and *D*). The signal of mCOUP-TFI was weaker than mCOUP-TFII in the motor neurons, whereas it was stronger than mCOUP-TFII in the rest of the spinal cord. mCOUP-TFI and -II expression disappeared from the somatic motor neurons at 16 days p.c. and persisted longer in the lateral horn, which contains sympathetic neurons (data not shown). In addition, the substantia gelatinosa in the dorsal horn expressed mCOUP-TFII at the cervical level between 14.5 and 18.5 days p.c. (Fig. 3 *D* and *F*). By 18.5 days p.c., mCOUP-TFI was expressed homogeneously in the entire spinal cord (Fig. 3*E*).

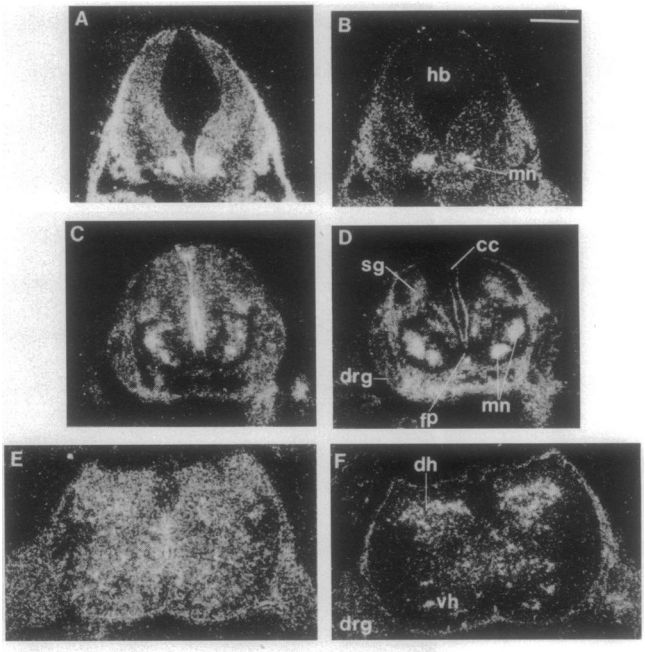

FIG. 3. Expression of mCOUP-TFI (*A*, *C*, and *E*) and -II (*B*, *D*, and *F*) in developing hindbrain and spinal cord. (*A* and *B*) Horizontal sections of 11.5-day p.c. embryo at the hindbrain (hb) level. mCOUP-TFII is expressed at high levels in the motor neurons (mn), while the expression of mCOUP-TFI is more uniform throughout the hindbrain neuroepithelium. (*C* and *D*) Horizontal sections of 14.5-day p.c. embryo at the level of the cervical spinal cord. mCOUP-TFI is expressed in most regions of the spinal cord, while mCOUP-TFII expression is seen in motor neurons of the ventral horn and in the substantia gelatinosa (sg) of the dorsal horn (dh). cc, Central canal; drg, dorsal root ganglion; fp, floor plate. (*E* and *F*) Horizontal sections of 18.5-day p.c. embryo at the level of the cervical spinal cord. mCOUP-TFI (*E*) is homogeneously expressed in the entire spinal cord. In contrast, mCOUP-TFII (*F*) expression is restricted to the dorsal horn. vh, Ventral horn. (Bar = 0.5 mm.)

DISCUSSION

The present study reports the spatiotemporal expression patterns of two mCOUP-TF genes in the developing CNS. Our results show that mCOUP-TFI and -II are specifically expressed in several regions during development of the brain. In the diencephalon, mCOUP-TFI and -II expression is restricted by the neuromeric boundaries.

The developing diencephalon displays a repeating set of bulging structures called neuromeres (20, 24, 25). These diencephalic neuromeres are developmental compartments established through restricted cell lineage. Each represents a primordium of a well-defined adult structure distinct from one another (20, 26). When the diencephalic neuromeres become conspicuous, mCOUP-TFI is characteristically expressed in the D1/D2 region, whereas mCOUP-TFII is primarily found in the D1 region. Such a segment-specific expression is first detected at 10.5 days p.c., at which time D1/D2 segmentation is already histologically apparent (27). The earliest axonogenesis further precedes this stage in this area. Within the area ranging between the midbrain and future D2, axonal tract primordia are already seen at 9.0 days p.c., well before the D1/D2 segmentation is formed (28). Segment-related expression patterns of mCOUP-TFs become clearer at 14.5 days p.c. onward, with the down-regulation of both genes in the pretectal area and COUP-TFII in D2 (Fig. 2 *E* and *F*). At present, it seems more reasonable to assume that mCOUP-TFI and -II may be among the genes that are involved in maintenance of neuromeric compartments and/or maintenance of segment-specific neuronal dif-

ferentiation rather than the establishment of segmentation of the forebrain itself or regulation of neurogenesis.

The secondary restriction of mCOUP-TF expression domains during development suggests that the genes may be regulated by cell lineage-related factors. A similar sharpening of expression boundaries has been reported for *GhoxB1* (previously *Ghox-2.9*), which becomes restricted to rhombomere 4 (29). Besides COUP-TF genes, several other homeobox genes (21, 30–33), *Pax* genes (34, 35), and members of the *Wnt* gene family (36, 37) are also expressed in a segment-restricted fashion in the diencephalon. Developmental fates of diencephalic neuromeres may be governed and maintained by these clonally regulated transcription factors or growth factors.

The differential expression patterns of mCOUP-TFI and -II in the CNS suggest that they may play different roles in neural development. However, the binding activities of COUP-TFI and -II in human and mouse are very similar *in vitro* (refs. 11 and 14; unpublished observations). This raises the question of how each COUP-TF functions in a specific manner. When expressed in different tissues, each COUP-TF might control different sets of genes and be involved in different pathways, resulting in different phenotypes. Even when expressed in the same tissues, they may exert different effects on downstream genes through their less conserved N-terminal domains, which have been shown to be responsible for cell- and receptor-specific activities of other superfamily members (38–40). Furthermore, in regulation of a target gene, the ratio of different transcription factors could also be crucial. Finally, the availability of the ligand for COUP-TFs could add another level of complexity to its activation capacity.

In addition, COUP-TFs could regulate the CNS development through their repression function on other members of the steroid/thyroid receptor superfamily. COUP-TFs bind to AGGTCA direct repeats and palindromes with various spacings (11). These include response elements for vitamin D_3 receptor (VDR), thyroid hormone receptor (TR), retinoic acid receptors (RARs), and retinoid X receptors (RXRs) (11, 13, 14). In cultured cells, COUP-TFs can repress VDR, TR, RAR, and RXR activities by competition for DNA binding (11–14) and by heterodimerization with RXR, which decreases the effective concentration of available RXR to form dimers with RAR, TR, and VDR (12, 13). Thus, COUP-TFs could also exert their function via controlling or modulating the retinoid and thyroid hormone pathways. To discriminate between these possibilities, and in view of the widespread and high-level expression of these two receptors during CNS development, the transgenic mouse system can be used to over-, under-, and ectopically express COUP-TFs.

We wish to thank Maya Dajee, Bernard Allan, Meijin Chu, and Lou Ann Stanley for their technical assistance. We would also like to thank Jane Lauder and Michael Figdor for valuable discussions. S.K. was supported by the Muscular Dystrophy Association. This work is supported by National Institutes of Health Grant DK 45641.

1. Miyajima, N., Kadowaki, Y., Fukushige, S., Shimizu, S., Semba, K., Yamanashi, Y., Matsubara, K., Toyoshima, K. & Yamamoto, T. (1988) *Nucleic Acids Res.* **16,** 11057–11074.
2. Wang, L.-H., Tsai, S. Y., Cook, R. G., Beattie, W. G., Tsai, M.-J. & O'Malley, B. W. (1989) *Nature (London)* **340,** 163–166.
3. Ritchie, H. H., Wang, L.-H., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1990) *Nucleic Acids Res.* **18,** 6857–6882.
4. Wang, L.-H., Ing, N. H., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1991) *Gene Expression* **1,** 207–216.
5. Ladias, J. A. A. & Karathanasis, S. K. (1991) *Science* **251,** 561–565.
6. Mlodzik, M., Hiromi, Y., Weber, U., Goodman, C. S. & Rubin, G. M. (1990) *Cell* **60,** 211–224.
7. Fjose, A., Nornes, S., Weber, U. & Mlodzik, M. (1993) *EMBO J.* **12,** 1403–1414.
8. Lutz, B., Kuratani, S., Cooney, A. J., Wawersik, S., Tsai, S. Y., Eichele, G. & Tsai, M.-J. (1994) *Development* **120,** 25–36.
9. Matharu, P. J. & Sweeney, G. E. (1992) *Biochim. Biophys. Acta* **1129,** 331–334.
10. Chan, S. M., Xu, N., Niemeyer, C. C., Bone, J. R. & Flytzanis, C. N. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10568–10572.
11. Cooney, A. J., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1992) *Mol. Cell. Biol.* **12,** 4153–4163.
12. Cooney, A. J., Leng, X., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1993) *J. Biol. Chem.* **268,** 4152–4160.
13. Kliewer, S. A., Kazuhiko, U., Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A. & Evans, R. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1448–1452.
14. Tran, P., Zhang, X.-K., Salbert, G., Hermann, T., Lehmann, J. M. & Pfahl, M. (1992) *Mol. Cell. Biol.* **12,** 4666–4676.
15. Eichele, G. (1989) *Trends Genet.* **5,** 246–251.
16. Kessel, M. (1992) *Development* **115,** 487–501.
17. Kessel, M. & Gruss, P. (1991) *Cell* **67,** 89–104.
18. Tata, J. R. (1993) *Bioessays* **15,** 239–248.
19. Wilkinson, D. G., Bailes, J. A., Champoin, J. E. & McMahon, A. P. (1987) *Development* **99,** 493–500.
20. Figdor, M. & Stern, C. (1993) *Nature (London)* **363,** 630–634.
21. Bulfone, A., Kim, H.-J., Puelles, L., Porteus, M. H., Grippo, J. F. & Rubenstein, J. L. R. (1993) *Mech. Dev.* **40,** 129–140.
22. Windle, W. F. (1932) *J. Comp. Neurol.* **55,** 99–138.
23. Windle, W. F. (1933) *J. Comp. Neurol.* **58,** 643–723.
24. Keyser, A. (1972) *Acta Anat.* **83,** Suppl. 59, 1–178.
25. Orr, H. (1887) *J. Morphol.* **1,** 311–372.
26. Puelles, L., Amat, J. A. & Martinez de la Torre, M. (1987) *J. Comp. Neurol.* **266,** 247–268.
27. Niimi, K., Harada, I., Kusaka, Y. & Kishi, S. (1962) *Tokushima J. Exp. Med.* **8,** 203–238.
28. Easter, S. S., Jr., Ross, L. S. & Frankfurter, A. (1993) *J. Neurosci.* **13,** 285–299.
29. Sundin, O. & Eichele, G. (1990) *Genes Dev.* **4,** 1267–1276.
30. Porteus, M. H., Bulfone, A., Ciaranello, R. D. & Rubenstein, J. L. R. (1991) *Neuron* **7,** 221–229.
31. Price, M., Lemaistre, M., Pischetola, M., Lauro, R. D. & Duboule, D. (1991) *Nature (London)* **351,** 748–751.
32. Robinson, G. W., Wray, S. & Mahon, K. A. (1991) *New Biol.* **3,** 1183–1194.
33. Simeone, A., Acampora, D., Gulisano, M., Stornaiuolo, A. & Boncinelli, E. (1992) *Nature (London)* **358,** 687–690.
34. Goulding, M. D., Chalepakis, G., Deutsch, U., Erselius, J. R. & Gruss, P. (1991) *EMBO J.* **10,** 1135–1147.
35. Walther, C. & Gruss, P. (1991) *Development* **113,** 1435–1449.
36. McMahon, A. P., Joyner, A. L., Bradley, A. & McMahon, J. A. (1992) *Cell* **69,** 581–595.
37. Roelink, H. & Nusse, R. (1991) *Genes Dev.* **5,** 381–388.
38. Bocquel, M. T., Kumar, V., Stricker, C., Chambon, P. & Gronemeyer, H. (1989) *Nucleic Acids Res.* **17,** 2581–2595.
39. Folkers, G. E., van der Leede, B. M. & van der Saag, P. T. (1993) *Mol. Endocrinol.* **7,** 616–627.
40. Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E. & Chambon, P. (1989) *Cell* **59,** 477–487.