Induction of the Intrinsic Enhancer of the Human Ciliary Neurotrophic Factor Receptor (CNTFRA) Gene by the TR4 Orphan Receptor

A MEMBER OF STEROID RECEPTOR SUPERFAMILY*

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A conserved hormone response element, CNTFR-DR1 (5′-AGGGCAAGGTCAAGG-3′), has been identified in the 5th intron of the α component of the ciliary neurotrophic factor receptor (CNTFRA) gene for the human TR4 orphan receptor (TR4). Electrophoretic mobility shift assays showed a specific binding with high affinity (Kd = 0.066 nM) between TR4 and the CNTFR-DR1. A reporter gene assay using chloramphenicol acetyltransferase demonstrated that the 5th intron of CNTFRA has an enhancer activity which could be induced by TR4 in a dose-dependent manner. Furthermore, our in situ hybridization data showed that abundant TR4 transcripts were detected in adult brain, in regions of cortical and hippocampal neurons, as well as in many developing neural structures, including brain, spinal cord, ganglia (sympathetic and sensory), and neuronal epithelia (retinal, otic, olfactory, and gustatory). The striking similarities in the expression patterns of TR4 and CNTFRA in the developing and postnatal nervous systems further support the potential role of TR4 in neurogenesis. Collectively, these data suggest that the human CNTFRA gene could represent the first identified neural-specific gene induced by TR4.

Members of the steroid/thyroid hormone receptor superfamily are transcription factors that can bind to specific DNA sequences called hormone response elements (HRE)1 and thereby regulate the expression of their target genes (1). This superfamily includes receptors for steroid, thyroid, vitamin D, and retinoids, and a large number of orphan receptors whose cognate ligands are still unknown (1). These members have been grouped into three categories according to their binding preference to HREs, the palindromic half-site AGAACA is preferred for the binding by the androgen, glucocorticoid, mineralocorticoid, and progesterone receptors; the direct repeat with various spacing is preferentially recognized by the estrogen, thyroid, retinoic acid, retinoid X, vitamin D, and many orphan receptors; and the single half-site of AGGTCA preceded by two specific flanking nucleotides is favored by some members which bind as a monomer, such as the steroidogenic factor 1 (2), TR3 orphan receptor (TR3/NGFI-B/nur77 (3), and the thyroid receptor (4). Some receptors, i.e. thyroid hormone receptor and chicken ovalbumin upstream promoter-transcription factors (COUP-TFs), are shown to be promiscuous for binding to different arrangements of the AGGTCA half-site (4, 5).

The human and rat TR4 cDNAs were isolated from testis and hypothalamus by degenerative PCR cloning (6). The TR4 cDNA shows high homology in nucleotide sequence with the TR2 orphan receptor (TR2; another orphan receptor isolated from our laboratory (7)), suggesting that these two orphan receptors constitute an unique subfamily within the steroid receptor superfamily. TR4 mRNA are widely expressed in the adult rat brain (6). Within the supraoptic nucleus, TR4 is one of the most abundant steroid receptors expressed there with the order thyroid hormone receptor > COUP-TFI > TR4 = COUP-TFII (8). Despite the high abundance of the TR4 transcripts in nervous tissues, the physiological function of TR4 in neurogenesis remains unclear.

To understand the role of TR4 in neurogenesis, we sought downstream regulatory events, namely target genes, which can be regulated by TR4. Thus, we examined the TR4 expression pattern during mouse embryogenesis to further confirm its participation in neuronal development, and then used this information to search for TR4 target genes involved in a neuronal-specific program. We then sought potential TR4 target genes based on the binding preference of TR4. In vitro binding assays suggested that TR4 is capable of binding to 5′-AGGTCA direct repeats with 1–6-base pair spacing (DR1-DR6).2 We hypothesized that the α component of ciliary neurotrophic factor receptor (CNTFRA) with DR1 in its 5th intron may be a target gene for TR4.

Ciliary neurotrophic factor (CNTF) is a member of the cytokine superfamily. It utilizes a three-component receptor system consisting of an extracellular CNTF-binding protein, known as CNTFRA (9), as well as two signal transducing β receptor subunits, gp130 and LIFRβ, which it shares with its cytokine relatives (10, 11). CNTFRA is bound to the cell membrane by a glycosylphosphatidylinositol anchor and its major function is to convey ligand-specificity (9). However, upon certain stimula-

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‡The abbreviations used are: HRE, hormone response element; CNTFRA, a receptor of ciliary neurotrophic factor; TR2, TR2 orphan receptor; TR3, TR3 orphan receptor; TR4, TR4 orphan receptor; Coup-TF1 and II, chicken ovalbumin upstream promoter-transcription factor I and II; RAR, retinoic acid receptor; RXR, retinoid X receptor; CNTF, ciliary neurotrophic factor; CNTFRA-BS, the 5th intron of CNTFRA gene; KT-PCR, reverse transcription-polymerase chain reaction; DR1, direct repeat with one spacing; TERE, thyroid hormone response element; Nbre, TR3/NGFI-B/nur77 response element; mTR4N, mouse N-terminal TR4 cDNA; EMSA, electrophoretic mobility shift assay; CAT, chloramphenicol acetyltransferase; E16, embryonic day 16.

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tion, CNTFRα can also be released from its glycosylphosphatidylinositol anchor and mimics the effect of CNTF on target gene activation (12). The expression of CNTFRα is mostly restricted to nervous tissues and is detected in all neurons that have been shown to respond to CNTF (13–16). Clinically, CNTF effectively protects against motor neuron degeneration in human Parkinson’s disease (17). The trophic effect of CNTF is also observed on the denervated skeletal muscle (18). Consistent with the CNTF effect on motor neurons, the essential function of the CNTFRα gene has been proved in mice lacking CNTFRα, which exhibit profound motor neuron deficits at birth (19). Despite the important function of CNTFRα, the regulation of the CNTFRα gene remain almost completely unknown.

Because little is known about TR4 targets, we focused study here on a DR1 element present in CNTFRα which can be potentially regulated by TR4. Thus, the 5th intron of the CNTFRα gene (CNTFR-I5) was cloned to show the DR1 response element, where TR4 binds, is conserved and functional. The consequence for TR4 interaction with this DR1 in its natural gene context was determined using a reporter gene assay. Moreover, the expression pattern of TR4 during mouse embryogenesis was examined by in situ hybridization and then compared to that of CNTFRα. Through these studies, our results suggest TR4 may function as an inducer in CNTFRα gene regulation and this event could happen in vivo. Thus, this study provides the first evidence for TR4 to interact with the neurocytokine signaling pathway during neurogenesis.

MATERIALS AND METHODS

Cloning of the 5th Intron of CNTFRα Gene—To clone the DNA fragment containing the 5th intron of the CNTFRα gene, polymerase chain reaction (PCR) was employed. Two oligonucleotides (E5: 5'-CAC-CTTCAATGTGACTGTGC-3', E6: 5'-GTGATGACTGACATGGCG-3') located in the coding regions flanking the 5th intron of human CNTFRα were synthesized for PCR amplification. The positions of E5 and E6 primers are 417–436 and 510–491, respectively (20). Purified genomic DNA (1 μg) isolated from several cell lines (human 1299 and Chinese hamster ovary) and from mouse tail were used as the templates. The following thermal cycling program was used to amplify the DNA fragment with the expected size around 270 base pairs: denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, for 35 cycles. The amplified DNA fragments (named as CNTFR-I5) from various template sources were gel purified and ligated into pT7 blue vector (Novagen). Sequence analysis using the Sequenase kit (U.S. Biochemical Corp.) confirmed the identity of these fragments.

Reporter Constructions—To test for enhancer activity, pCAT-promoter vector (Promega), which contains an SV40 promoter upstream (+) located in the coding regions flanking the 5th intron of human CNTFRα were synthesized for PCR amplification. The positions of E5 and E6 primers are 417–436 and 510–491, respectively (20). Purified genomic DNA (1 μg) isolated from several cell lines (human 1299 and Chinese hamster ovary) and from mouse tail were used as the templates. The following thermal cycling program was used to amplify the DNA fragment with the expected size around 270 base pairs: denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, for 35 cycles. The amplified DNA fragments (named as CNTFR-I5) from various template sources were gel purified and ligated into pT7 blue vector (Novagen). Sequence analysis using the Sequenase kit (U.S. Biochemical Corp.) confirmed the identity of these fragments.

Scatchard Analysis—The DNA-protein binding assay was performed as described previously with modifications (22). Briefly, 0.05 μl of in vitro translated TR4 was incubated with various concentrations of the probe. Protein-DNA complexes were resolved on a 5% non-denaturing polyacrylamide gel (4°C) in 0.3 M Tris base, 0.1 M glycine, and 10 mM EDTA. After exposure to a film to visualize the free probe and protein-probe complex, the respective bands were excised and counted directly in a scintillation counter. The dissociation constant (Kd) value and Bmax were calculated using the EBDA program (Biosoft).

Cell Culture, Transfection, and CAT Assay—Chinese hamster ovary cells were cultured and transfected by the calcium phosphate coprecipitation procedure as described previously (22). To normalize the transfection efficiency, the pCMVβ (Clontech) was co-transfected. Results were plotted as the mean ± S.D. of at least three independent experiments of CAT expression normalized to β-galactosidase activity.

Cloning Mouse TR4 cDNA—To clone the full-length mouse TR4 cDNA, reverse-transcription followed by PCR (RT-PCR) was employed, following the standard protocol recommended by the manufacturer (Perkin-Elmer). Mouse testis total RNA (1 μg) was used as a template. The N- and C-terminal mouse TR4 cDNA fragments were separately amplified using the primer sets TR4-16 and TR4-15 or TR4-3 and TR4-42, respectively. The primer sequences are as followings: TR4-16 5'-GCAGACTCA-CACAGAAGATTGTCG-3' and TR4-15 5'-CAACAGCTACAGACGACCTC-3', TR4-3 5'-CTCAGAGATCTATTCCG-3', and TR4-42 5'-CAACAGCTACAGACGACCTC-3'.

FIG. 1. The genomic position and nucleotide sequence of the 5th intron of the CNTFRα gene. A, the intron-exon structure of the cytokine receptor-like domain of CNTFRα gene. The introns are indicated by roman numerals. The position of the 5th intron is indicated by an asterisk (*). B, the alignment of the nucleotide sequence of CNTFR-I5 homologues between hamster, mouse, and human. Dashes indicate identity and dots represent gaps. The brackets mark the junction of 5th intron with its neighboring exons. The box outlines the CNTFR-DR1 response element. The 5'-AGGGTCA-like sequences are underlined. TRE-like and consensus NRE sequences are as indicated. The sequence and orientation of E5 and E6 primers are as shown.
The identity of PCR products was confirmed by sequencing and showed high homology to human counterparts. These two cDNA fragments were then ligated together to produce a full-length mouse TR4 cDNA.

Probe Preparation—The N terminus of the mouse TR4 cDNA was cloned by RT-PCR. The primers TR4-16 and TR4-23 (5'-ACACAGTACTCTACCACCTG-3') were used to amplify a PCR product (around 400 bp in size) from mouse testis total RNA, which was cloned into the T7 blue vector (Novagen), confirmed by DNA sequencing (U. S. Biochemical Corp.), subcloned into pBluescript SK+ (Stratagene), and designated as mTR4N-pBS. For Northern blot analysis, radiolabeling was performed using a PCR reaction including primers (TR4-16 and TR4-23), 0.1 ng of linear template (mTR4N-pBS plasmid), 5 nmol of each dATP, dGTP, and dTTP, and 0.1 μCi of [α-32P]dCTP (DuPont). For in situ analysis, radiolabeled antisense and sense TR4 probes (specific activity 1–2 × 10^6 cpm/μg) were generated by in vitro transcription as described previously (22).

Northern Blot Hybridization—Total RNAs from selected mouse tissues were isolated, electrophoresed, and transferred onto a nylon membrane as described previously (6). The blot was hybridized with the mouse mTR4N probe, washed, dried, exposed to PhosphorImager intensifying screen for 16 h. The image was scanned and printed.

In Situ Hybridization Analysis—Embryo collection, section preparation, and in situ hybridization were performed as described previously (22). Both the antisense and sense TR4 probes were included for each batch of experiments. Washes were performed with high stringency (2 × SSC, 50% formamide at 65 °C (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0)) before and after RNase digestion (20 mg/ml for 30 min). Slides were dipped into Kodak NTB2 emulsion and exposed for 2 weeks. Subsequently, slides were developed in Kodak D19 developer, fixed, dehydrated, and mounted for dark field analysis. Some slides were stained with hematoxylin for light-field analysis.

RESULTS

Cloning of the 5th Intron of the CNTFRα Gene—Based on the genomic organization and DNA sequence published by Valenzuela et al. (20), the 5th intron of the human CNTFRα gene (CNTFR-I5) contains one perfect DR1 (5'-AGGTCAACAGTCA) and four copies of AGGTCA-like half-sites. It is especially amazing because so many AGGTCA-like sequences are simultaneously localized within a very small intron of around 176 base pair. We wondered whether some of these sites might be
HREs regulated by steroid receptors. As shown in Fig. 1A, CNTFR-I5 is located in the middle of the coding sequence for the first fibronectin-like subdomain within the cytokine receptor-like domain of the CNTFRα gene (Fig. 1A) (20). The presence and positions of introns in the cytokine receptor-like domain are conserved among members in the cytokine receptor superfamily (20), implying CNTFR-I5 may contribute a regulatory role through these response elements. To test this hypothesis, we cloned the CNTFR-I5 from human, hamster, and mouse by PCR (see “Materials and Methods”). Sequence comparisons between these three species showed that the DR1 and putative thyroid hormone receptor-binding site (TRE-like sequence) (4) were conserved but the TR3/NGFI-B/nur77-binding site (3) has one mismatch at its end (Fig. 1B). The other two AGGTCA-like sequences were not well conserved. The appearance of three different types of highly conserved HREs within a small intron suggests that CNTFR-I5 may be critical for CNTFRα gene regulation.

TR4 Binds Specifically to the CNTFR-DR1 with High Affinity—To examine whether CNTFR-DR1 could bind TR4, we carried out in vitro DNA binding experiments. Gel retardation assays were performed with in vitro translated TR4 using the 32P-labeled CNTFR-DR1 oligonucleotide as a probe. As shown in Fig. 2A, a specific DNA-protein complex was formed in the presence of both probe and TR4 (lane 3, hollow arrow) but was absent in the reaction containing probe and the mock-translated control (lane 2). This TR4-CNTFR-DR1 complex could be essentially abolished by as low as a 10-fold molar excess of unlabeled CNTFR-DR1 oligonucleotide (lane 4), but remained intact in the presence of a 100-fold molar excess of “mutant” CNTFR-DR1 oligonucleotide (lane 6). Moreover, this retarded complex could be fully supershifted in the presence of the anti-TR4 monoclonal antibody (lane 8, solid arrow). As a negative control, an unrelated monoclonal antibody with the same IgG2 isotype as that of TR4 showed no effect on the retarded complex (lane 7). Together, these data indicate that the CNTFR-DR1 is a specific binding site for TR4.

To determine the binding affinity of TR4 and CNTFR-DR1, we performed Scatchard analysis by EMSA. The typical EMSA pattern of protein-DNA complex formed between increasing amounts of CNTFR-DR1 probe (0.0039–2 ng) and fixed amounts of TR4 was shown (Fig. 2B). The radioactivity of specific complex (bound) and unbound (free) probe were quantitated for the subsequent Scatchard plot analysis. The results are consistent with a single binding population for the specific DNA-protein complex with a dissociation constant (Kd) of 0.066 nM and Rmax of 0.089 nM (Fig. 2C). This binding affinity is about 15–45-fold higher than the Kd range for steroid receptors and their HREs (4). At very low probe concentrations, the specific protein-DNA complex was still visible (Fig. 2B, lanes 2–4). That was further consistent with the calculated high affinity dissociation constant.

Enhancer Activity of CNTFR-I5 Induced by TR4—To investigate whether TR4 could regulate the CNTFRα gene expression through interaction with CNTFR-I5, we carried out the CAT assay following co-transfection of expression vectors and CAT reporter constructs into Chinese hamster ovary cells. Three enhancer-reporter plasmids were created in the present study to test whether the enhancer activity of CNTFR-I5 is position- or orientation-dependent (Fig. 3A). As shown in Fig. 3B, in the presence of the CNTFR-I5, TR4 induced transcriptional activity up to 15–30-fold in a dose-dependent manner (compare lanes 3 and 4 to lane 2; lanes 8 and 9 to lane 7; lanes 13 and 14 to lane 12). In contrast, this induction did not occur when the antisense TR4 expression vector was transfected (lanes 5, 6, 10, 11, 15, and 16). Different orientations or positions did not appear to affect the TR4-mediated transcriptional activity. TR4 does not significantly induce the CAT reporter in the absence of the DR1(22). Taken together, these results suggest that TR4 might induce CNTFRα gene expression via the CNTFR-I5 enhancer.

The N-terminal Mouse TR4 Probe Is Highly Specific for Northern Blot and in Situ Hybridization Analyses—The entire coding region of mouse TR4 cDNA was cloned by RT-PCR (for details see “Materials and Methods”) (Fig. 4A). Sequence comparison with other members of the steroid receptor superfamily indicated that the N-terminal mouse TR4 cDNA fragment (mTR4N) covered between primer TR4-16 and TR4-23 is the most divergent; the homology within this region is around 30% at nucleotide level between mouse TR4 and TR2. To test the specificity of mTR4N, total RNA samples isolated from mouse

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testis and kidney were used as positive and negative controls, respectively. As shown in Fig. 4, the mTR4N probe hybridized to two bands, 7.8 and 2.8 kilobase in size, in mouse testis (Fig. 4B, lane 1). The sizes of these two TR4-hybridized bands are different from those of TR2 transcripts, which are 2.4 (major band) and 2.9 kb (minor band) in size. No hybridization signal was detected in the kidney (Fig. 4B, lane 2). These data are consistent with previous observations (6) supporting that the mTR4N probe is specific and only hybridizes to TR4 transcripts.
and dorsoventral axes (Fig. 6, B and C, ne). Subsequently, during E14-E16, expression became restricted to the ventricular zones of brain vesicles, where cells are rapidly proliferating (23) (Fig. 6, D-F, arrow and arrowhead). Sense mTR4N probe did not detect specific signal in E14 embryo (Fig. 6A). Intensive TR4 transcripts in E16 brain were also observed in the striatum (Fig. 6F, Sr), active dividing population of the cerebellar primordium (Fig. 6E, cp), and spinal cord (Fig. 6D, sp). The spinal motor neurons (Fig. 6D, m) were also positive for TR4 expression. This expression pattern is consistent with a potential role for TR4 in the proliferation and/or early differentiation of neuronal precursors within the central nervous system. During the development of the peripheral nervous system, TR4 transcripts were also actively expressed within the ganglia and the neural epithelium of many sensory organs. Abundant TR4 transcripts were detected in the dorsal root (Fig. 6, C-E, dg), superior cervical (Fig. 6, G-H, c), sympathetic (Fig. 6H, s), and trigeminal (Fig. 6, G, V) ganglia. Its abundance within the dorsal root ganglia through E11-E16 suggests a role for TR4 through the progression of these neurons from precursors to postmitotic neurons (24). High levels of TR4 mRNA were also found within all targets of sensory innervation, including the neuronal epithelium of inner ear (Fig. 6, G, e), nasal cavity (Fig. 6, J, oe), retina (Fig. 6f), pal (14), retinal (nuclear and ganglion layers) (15), and motor (neostriatum and spinal motor) neurons (16), as well as the sympathetic (superior cervical) and sensory (trigeminal and dorsal root) (25) ganglia. Its abundance within the developing dorsal root ganglia and spinal cord during E11-E15 of rat embryos with its transcripts restricted in the mitotically active populations (25). Our in situ data clearly show that TR4 have a similar expression pattern in E9-E14 mouse embryos (correlate to E10-E15 rat embryos) and our RT-PCR assay (not shown) show that TR4 transcripts are present as early as E9.

Several tissues expressing abundant TR4 transcripts are also known to respond to CNTF. Examples are the hippocampal (14), retinal (nuclear and ganglion layers) (15), and motor (neostriatum and spinal motor) neurons (16), as well as the sympathetic (superior cervical) and sensory (trigeminal and dorsal root) (25) ganglia. Since CNTFRa is essential for the motor neuron development (19), our in situ data showing abundant TR4 transcripts there indicate that TR4-mediated CNTFRa induction could be an important process for motor neuron survival. An interesting coincidence is that the neurotransmitter dopamine has been proposed as an activator for TR2 and, possibly, for TR4 (26). Assuming dopamine activates TR4 which may then induce CNTFRa expression, it is possible that the reduced dopamine level, in the case of Parkinson’s disease, can affect the activation of TR4 and CNTFRa. Thus, a higher CNTF concentration may be needed to maintain neuron integrity. This hypothesis reasonably explains the action of CNTF and proposes TR4 as a bridge between the dopamine and the CNTF signal transduction pathways.

Outside the developing nervous system, both TR4 and...
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During the adult stage, TR4 and CNTFRα transcripts are co-expressed within neuron cell bodies of many brain regions, including cortex, hippocampus, and cerebellum. Outside the adult brain regions, whether both are co-expressed is unknown.

TR4 Induces Transcriptional Activation via a DR1 Located within CNTFR-I5—The DR1 present in CNTFR-I5 represent the first intronic inducible DR1 response element regulated by TR4. Using in vitro binding assay, a DR1 sequence has been shown to be the high affinity binding site for several steroid receptors, including RARs (28), RXRs (29), peroxisome proliferator-activated receptor (30), COUP-TFs (31), TR2 (22), and TR4.2 For certain gene regulation to occur, these DR1-binding factors may compete with each other for the binding site depending on the affinity and the relative abundance. The relative affinity among these DR1 binding factors are unclear. However, our data show a very high affinity of TR4 binding to the CNTFR-DR1, indicating once TR4 is present, it could dominate over other DR1 binding factors for occupying the DR1 response element.

Upon binding to a DR1 present in the promoter, these DR1-binding steroid receptors or their complexes may either activate or repress gene transcription in reporter gene assays. For example, RAR/RXR heterodimer mediates retinoic acid response on phosphoenolpyruvate carboxykinase gene induction (28). Peroxisome proliferator-activated receptor alone or complexed with RXR is able to activate the acyl-CoA oxidase gene expression (30). Although COUP-TFs were originally identified as required factors for ovalbumin gene transcription (32), in contrast, they repress retinoic acid induced transactivation on a DR1 reporter construct (33). Similar phenomena are also observed for TR2 (22) and TR4 on a DR1-mediated repression of the retinoic acid-induced CRBPII promoter activity. As opposed to the repression effect of TR4 on the CRBPII promoter,2 our data demonstrate that TR4 could bind to a DR1 sequence and induce transcriptional activation (Fig. 2, 3). The effects of other DR1-binding factors on CNTFR-I5 have not been examined yet. These results indicate that through the binding to a similar DR1 sequence, whether the transcriptional activity is up or down-regulated may depend on the receptor, gene context, response element, and the assay system used. Other studies also have made similar observations (5).

TR4 may be able to interact with other DR1-binding proteins, such as RARα, RARβ, RXRγ, COUP-TFI, COUP-TFII, and TR2, and exert combinational effects on CNTFRα gene regulation. Among these DR1-binding protein, RARα (34), RARβ (34), and RXRγ (35) are detected in the more differentiated neuronal and oral cavities (J). Ventricular zone was indicated as arrowheads (F). Abbreviations used are: c, superior cervical ganglion; cp, cerebellum primordium; cx, cerebral cortex; dp, dorsal root ganglia; e, otic epithelium; g, ganglion layer of retina; l, lens; m, motor neuron; ms, muscle; n, nuclear layer of retina; ne, neural epithelium; oe, olfactory epithelium; r, retina; s, sympathetic ganglia; sp, spinal cord; Sr, striatum; t, tongue epithelium; V, trigeminal ganglion. The size bars represent 1 and 100 nm for A-E and F-J, respectively.

FIG. 6. TR4 mRNA expression during mouse embryogenesis. Sagittal sections of embryos were hybridized with either sense (A) or antisense mTR4N riboprobe (B-J) and photographed under dark-field illumination. A–E, low-magnification photographs showed the TR4 expression patterns of embryos with the development stages indicated at the top. The restricted expression of TR4 transcripts to the ventricular zones were indicated as arrows (D) and arrowhead (E). F–G, high-magnification photographs showed TR4 hybridization signals in sections of an E16 embryo within regions of the developing forebrain (F), inner ear (G), spinal cord (H), eye (I), and the junction between nasal...
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types, which is consistent with the roles of retinooids in neuron differentiation and their expression patterns do not match that of CNTFRα. Since TR4, TR2, COUP-TFI, and COUP-TFII (33) are localized mainly in the actively mitotic neuron populations which also express CNTFRα, the chance for them to regulate CNTFRα transcription is much higher. Especially, COUP-TFI has been demonstrated to be involved in spinal motor neuron development in chicken (36). How to coordinate these DR1-binding proteins for the regulation of CNTFRα will be an intriguing question to follow.

In addition, the presence of several 5′-AGGTCA-like half-sites within CNTFR-I indicate some steroid receptors which bind as a monomer may regulate the CNTFRα expression. The candidates include thyroid hormone receptor and TR3/NGFI-B/nur77. This idea is supported by their localization in adult brain (8, 37). However, much lower protein-DNA affinity between these receptors and the half-site may not support these factors that would play major roles on CNTFRα regulation.

TR4 Acts as an Activator—In addition to the roles of TR4 in restricting other hormonal signaling pathways2 and in inhibiting the SV40 promoter (21), our data here suggest that TR4 can also function as a transcriptional activator with high induction activity through CNTFR-I. This TR4-mediated transcriptional activity is consistent as tested in Chinese hamster ovary or P19 cell lines (data not shown). In contrast, TR4 repressed the retinoic acid-induced transcriptional activity through a perfect DR1 response element located in its 5th intron. In addition, the expression patterns of both genes correlated well during the developmental and postnatal neurogenesis. These findings may extend our study to investigate the interactions between steroid receptors and cytokine/cytokine receptors.

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