The Human RC3 Gene Homolog, NRGN Contains a Thyroid Hormone-Responsive Element Located in the First Intron*

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ABSTRACT

NRGN is the human homolog of the neuron-specific rat RC3/neurogranin gene. This gene encodes a postsynaptic 78-amino acid protein kinase substrate that binds calmodulin in the absence of calcium, and that has been implicated in dendritic spine formation and synaptic plasticity. In the rat brain RC3 is under thyroid hormone control in specific neuronal subsets in both developing and adult animals. To evaluate whether the human gene is also a target of thyroid hormone we have searched for T₃-responsive elements in NRGN cloned genomic fragments spanning the whole gene. Labeled DNA fragments were incubated with T₃ receptors (T₃R) and 9-cis-retinoic acid receptors and immunoprecipitated using an anti T₃R antibody. A receptor-binding site was localized in the first intron, 3000 bp downstream from the origin of transcription. Footprinting analysis revealed the sequence GGATTAATGAGGTAA, closely related to the consensus T₃-responsive element of the direct repeat (DR4) type. This sequence binds the T₃R-9-cis-retinoic acid receptors heterodimers, but not T₃R monomers or homodimers, and is able to confer regulation by T₃R and T₃ when fused upstream of the NRGN or thyminode kinase promoters. The data reported in this work suggest that NRGN is a direct target of thyroid hormone in human brain, and that control of expression of this gene could underlay many of the consequences of hypothyroidism on mental states during development as well as in adult subjects. (Endocrinology 140: 335–343, 1999)

The calmodulin-binding and protein kinase C substrate, RC3¹/neurogranin, is a member of a family of proteins expressed in the central nervous system, for which the term calpain has been recently proposed (1–3). These proteins share many structural features, including a calmodulin-binding domain, and are highly conserved among species. RC3 is predominantly located in the soma, dendrites, and dendritic spines of neuron subsets in the cerebral cortex and hippocampal neurons (18, 19). Regulation of RC3 expression by thyroid hormone occurs in developing as well as adult animals (17) and correlates to the known morphogenetic actions of thyroid hormone on dendritic spines of cerebral cortex and hippocampal neurons (18, 19). Regulation by T₃ occurs in other species besides the rat. Thus, the goat RC3 homolog has also shown to be thyroid hormone dependent in vivo (20). In addition, in mouse-derived hypothalamic GT1–7 cells, thyroid hormone induces an increased RC3 expression, which occurs at the transcriptional level and is independent of new protein synthesis (21). RC3 regulation by T₃ is therefore presumably mediated through a direct interaction of thyroid hormone receptors with regulatory regions in the RC3 gene.

In brain as well as in other tissues, T₃ acts in the cell nucleus after binding to specific receptors. These receptors are members of a large family of transcription factors that includes the receptors for other hydrophobic ligands, such as retinoids, steroid hormones, and other hormones or metabolites. They control important developmental and physiological processes by modulating the expression of genes after binding to specific DNA sequences, known as response elements, that are usually located in the upstream promoter region of the target genes (22–24). Most of these nuclear receptors usually bind to DNA as homodimers or heterodimers with the 9-cis-retinoic acid receptor (RXR) (25). In the case of thyroid hormone, the response elements, or T₃REs, usually consist of a direct repeat of the consensus hexamer sequence, AGGTCA,
separated by four nucleotides (DR4), although other configurations are also relatively common (26, 27).

We have previously reported the identification, cloning, and determination of the structure of the human RC3 homolog gene, which we have named NRGN (28). The gene spans 12 kb and contains four exons and three introns. Its organization is identical to that of the rat Ng/RC3 gene, and alignment of protein sequences revealed a 97.5% identity. Besides characterization of the gene and chromosomal mapping, few data are available on NRGN expression in humans (29). We have observed that its expression in the monkey brain follows a pattern similar to that previously described for the rat or goat brain (results to be published). This suggests that NRGN could also be under thyroid hormone regulation in primates. To try to answer this question as well as to disclose the mechanism of regulation by T₃, we have searched for T₃REs in the human NRGN gene. Our previous studies failed to disclose any such regulatory elements in the 5′-flanking region of the rat or human genes (21, 28, 30). In the present work we have used an immunoprecipitation technique that allowed the screening of the whole gene. As a result we report on the localization and characterization of this study were synthesized as two complementary strands with 5′-GATCCAAGAGTTTGACTGGCTGATTTCCAGTTTGTG-3′.

DNA, cloned in the plasmid pSG-5 (supplied by Dr. H. Stunnenberg, University of Nijmegen, The Netherlands). The oligonucleotides used in this study were synthesized as two complementary strands with 5′-TCGACTTTCCAAAATGGGGATTACTTAAGGTAATATC 3′.

As we mentioned in the introduction, the T₃RE present in the long terminal repeat of the NRGN

**Materials and Methods**

**Plasmids and oligonucleotides**

The following plasmids were used in this study: pBLCAT-2 a chloramphenicol acetyltransferase (CAT) vector, in which the expression of the reporter enzyme is under control of the thymidine kinase promoter; thyroid hormone receptor (T₃R) expression vector, consisting of the complementary DNA encoding the rat T₃Rα-1 cloned in pCDM8 (a gift from Dr. P. R. Larsen, Brigham and Women’s Hospital, Boston, MA); and retinoid X receptor expression vector, the human RXRα complementary DNA, cloned in the plasmid pSG-5 (supplied by Dr. H. Stunnenberg, University of Nijmegen, The Netherlands). The oligonucleotides used in this study were synthesized as two complementary strands with 5′-extensions according to the sequence of the NRGN T₃RE, as shown in this paper, and the T₃Rα present in the long terminal repeat of the Moloney murine leukemia virus (Mo) (31). The sequences of these nucleotides were as follows (the DR4 half-sites are underlined): NRGN T₃RE, 5′-TCGACCTCCAAAAATGGGGATTAAATGAGGTAATATC 3′; and Mo T₃RE, 5′-TCGACAGGGTCATTTCAGGTCCTTGC-3′.

The nonspecific oligonucleotide (NS) used for the gel retardation experiments consisted of a 43-mer oligonucleotide corresponding to a region of the promoter of rat proteolipid protein with the following sequence: 5′-GATCCAAGAGTTGACTGGCTGATTTCCAGTTTGTG-3′.

The mutated oligonucleotides of the NRGN T₃RE were the following (the nucleotide changes are underlined): M1, 5′-TCGACCTCCAAAAATGGGGATTAAATGAGGTAATATC 3′; M2, 5′-TCGACCTCCAAAAATGGGGATTAAATGAGGTAATATC 3′; and M3, 5′-TCGACCTCCAAAAATGGGGATTAAATGAGGTAATATC 3′.

The labeled DNA used in the footprint assay was amplified using the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGACTTTCCCGATCAAC3′) and the internal primer of the NRGN fragment (1588: 5′-TGAC...
formamide loading buffer (80% formamide, 20 mM EDTA, 0.025% bromophenol blue, and 0.025% xylene-cyanol) and boiled for 3 min before loading on a 8% polyacrylamide-7 M urea sequencing gel. Electrophoresis was carried out at room temperature in 1X Tris-borate buffer at 250 V. The gels were dried and exposed to films.

Electrophoretic mobility shift assays (EMSA)

To study the interaction of T3R and RXR proteins with DNA, we used in vitro translated nuclear receptors. Complementary oligonucleotides were annealed, labeled with T4 polynucleotide kinase and 

\[ ^32P \]ATP, and purified with QIAquick Nucleotide Removal Kit (Qiagen, Chatsworth, CA). The translated receptors were incubated in 13 mM Tris base, 88 mM KCl, 20 mM HEPES (pH 7.9), 5 mM DTT, and 0.3 mg/ml poly(dI-dC)] at room temperature. After 15 min, labeled probes (4 x 10^6 cpm) were added to the binding mixture, and incubation was continued for 15 min. Competition gel-shift studies were carried out by adding the indicated amounts of competitor oligonucleotides at the same time as the probe. The protein-DNA complexes formed during binding reactions were separated on 6% polyacrylamide gels in 0.25 X Tris-borate buffer at 250 V. The gels were dried and exposed to films.

Supershift analysis was carried out by the addition of 1 µl of an anti-T3R antibody to the gel shift reaction after the 30-min incubation. The reaction mixture was incubated on ice for an additional 30 min and then loaded on the gel.

For off-rate experiments, a 200-fold molar excess of unlabeled oligonucleotides was added after the binding reaction. Samples were withdrawn at intervals between 0–45 min and directly loaded on a running gel. These experiments were performed in the presence of either 1 mM EDTA or 1 mM MgCl\(_2\) in the binding buffer (35).

Results

Detection of a T3R binding site in the first intron of the human neurogranin gene

Previous studies using genomic clones of the rat RC3 and human NRGN genes failed to show the presence of T3REs in the corresponding upstream promoter regions (28, 30). The fact that T3 induces expression of RC3 at the transcriptional level, both in vivo and in cultured cells (21, 36), and that the effect on cultured cells was relatively fast and independent of protein synthesis prompted us to analyze other regions of the gene in search of putative T3RE sequences. The human gene was used as starting material for these studies for two reasons: firstly for convenience, because we had genomic clones available from a previous study (28), and secondly for biological relevance, because the presence of a T3RE would strongly suggest that NRGN gene is also under T3 regulation.

We followed an immunoprecipitation technique to isolate genomic fragments able to bind the T3 receptor protein (34). NRGN genomic clones were cleaved by a set of restriction enzymes, followed by end labeling of the resulting DNA fragments. The labeled fragments were then used in coinmunoprecipitation assays in an attempt to identify T3REs on the basis of their expected interaction with the receptor. For immunoprecipitations, we used a mixture of T3R/RXR-programmed lysate proteins, previously obtained in vitro using a coupled transcription and translation rabbit reticulocyte lysate system. Control samples contained unprogrammed reticulocyte lysate. The mixture of labeled DNA fragments was incubated with either control or T3R/RXR-programmed lysate proteins, and the resulting complexes were immunoprecipitated with a specific anti-T3R antibody. Parallel incubations were performed in the presence of an excess of a DR4 T3RE, like the one present in the Moloney murine leukemia virus promoter, to determine the absence of nonspecific binding to DNA.

Figure 1A shows the structure of the human gene based on the report by Martinez de Arrieta et al. (28). The gene contains
four exons and three introns. Exon 1 encodes the 5'-untranslated region and the first five amino acids of the protein sequence; the rest of the NRGN protein is encoded in exon 2. Exons 1 and 2 are interrupted by an intron of about 5 kb long. In this work, we used several DNA fragments of sizes up to 1.5 kb, encompassing the human gene from about 3 kb upstream from the initiation of transcription to 0.5 kb downstream from the end of the fourth exon. All of these genomic fragments were negative in the immunoprecipitation assays, except for a 1.5-kb fragment of the first intron of the gene from approximately nucleotide 3000–4500 (Fig. 1A) from the origin of transcription. This fragment was cloned in pMosblue and subjected to further restriction digestion and immunoprecipitation analysis to more precisely delineate the T3R-interacting region. Figure 1A shows a restriction enzyme map, and Figure 1B shows the result of immunoprecipitation digestion with XbaI plus EcoRI produced four fragments: two of 0.1 kb, one of 1.4 kb, and one of 2.8 kb, the latter containing the vector. The labeled fragments were resolved in agar electrophoresis as three bands (Fig. 1B, lane D). After incubation with T3R/RXR plus an anti-T3R antibody (lane R), only the 1.4-kb band was precipitated. The specificity of this effect was confirmed by performing a similar coimmunoprecipitation experiment using as unlabeled competitor an excess of the T3RE present in the Moloney murine leukemia virus (lane Mo). In addition, the band was not observed if an unprogrammed reticulocyte lysate (lane RL) was used. Digestion with BamHI produced three bands, consisting of the vector plus two bands of 0.9 and 0.6 kb, respectively. After incubation with receptors and the anti-T3R antibody, only the 0.6-kb band was significantly immunoprecipitated. Further digestion and immunoprecipitation of smaller fragments (not shown) narrowed the size range of the DNA able to bind T3R/RXR to a 260-bp BamHI-EcoRV fragment derived from the 5’-portion of the original fragment.

These results strongly suggested the presence of a T3RE in the first intron of the human NRGN. Alignment of the sequences of the human and rat introns (37) showed a high degree of similarity in this region of the first intron, and preliminary immunoprecipitation experiments similar to those described above, but using the rat gene, suggested the presence of a T3RE in a similar position (not shown). To further delineate the receptor binding sequence, DNase I footprinting analysis were performed. The 260-bp BamHI-EcoRV fragment was subcloned in pMosblue, labeled by PCR in the lower strand, and used in the DNase I footprinting assay. Incubation of the fragment with nuclear extracts from HeLa cells expressing T3Rα and RXRα, plus increasing amounts of DNase I (Fig. 2A) resulted in a major protected sequence of 20 nucleotides. Control reactions performed in the presence of BSA showed no protected sequence. Analysis of the protected sequence shown in Fig. 2A reveals an imperfect direct repeat of the consensus half-site AGGTCA separated by four nucleotides (GGATTAAatgAGGTCA).

To analyze whether the sequence identified in the first intron as a putative T3RE was functional in vitro, trans-activation assays were performed (Fig. 2B). For this purpose, either a 140-bp fragment from the NRGN first intron containing the T3RE or a single copy of a double stranded oligonucleotide (NRGN T3RE, as shown in Materials and Meth-
ods) was cloned upstream from the thymidine kinase promoter in the pBLCAT-2 vector (140-tk-CAT or NRGN-tk-CAT, respectively). COS-7 cells were cotransfected with either of these two constructs together with expression vectors encoding T3R and RXR. For comparison, cells were similarly cotransfected with the CAT expression vector containing a single copy of the strong T3RE present in the Moloney murine leukemia virus long terminal repeat, a prototypical DR4 (Mo-tk-CAT). CAT activity was measured after incubating the cells in the absence or presence of T3. The cells transfected with either NRGN construct responded to the addition of T3 with an increased CAT activity (Fig. 2B, upper panel). In addition, we checked whether the T3RE was able to confer regulation in the context of the NRGN promoter. For this purpose, either the Moloney DR4 or the NRGN T3RE was inserted upstream of the NRGN promoter in the promoterless pBLCAT-3 vector (Mo-0.6-CAT and NRGN-0.6-CAT, respectively). In both constructs the addition of T3 increased CAT activity, whereas it did not when the 0.6-CAT construct without a T3RE was used as the control (Fig. 2B, lower panel).

**Interaction of the thyroid hormone receptor with the NRGN T3RE**

A set of experiments was designed to characterize the interaction of T3R in vitro with the NRGN T3RE by EMSA, using in vitro translated T3Rβ and RXRa, and labeled oligonucleotides. For comparison, we also used the Moloney T3RE (Fig. 3A). When labeled NRGN T3RE oligonucleotide was incubated with human T3Rβ and RXRa (lane 2), a retarded band was observed showing the same mobility shift than the RXR-T3R heterodimer obtained with the Mo T3RE (lane 1). Neither T3R (lanes 6 and 8) nor RXR (lane 9) alone bound the T3RE. The specificity of receptor binding was determined by the addition of an excess of unlabeled competitors. The addition of Mo and NRGN oligonucleotides (lanes 3 and 5) completely avoided formation of the retarded band. The complex was not competed, however, when incubated in the presence of a random DNA sequence (lane 4). Further proof of the binding of T3R to the NRGN oligonucleotide was obtained in supershift experiments, as shown in Fig. 3B. In these experiments, the same shifted band was produced with T3R/RXR plus either Mo (lane 10) or NRGN (lane 12) oligonucleotides. The NRGN band was supershifted by addition of an anti-T3R antibody to the incubation mixture (lane 11), whereas the antibody alone was without effect (lane 13). These experiments suggested that the NRGN T3RE bound the RXR-T3R heterodimer and had no affinity for either T3R monomer or homodimer.

To determine the relative binding strength of RXR-T3R heterodimer to NRGN T3RE compared with that of the Mo

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**Fig. 3.** Band shift assay demonstrating the binding of RXR-T3R heterodimer to the putative T3RE NRGN. A, Labeled oligonucleotides representing the NRGN T3RE (NRGN) and the DR4 thyroid hormone-responsive element of the Moloney murine leukemia virus (Mo) were incubated with in vitro translated T3Rβ and RXRa. Both elements were capable of forming a RXR-T3R heterodimer shift (lanes 1, 2, and 7). Competition assays were performed with a 200-fold molar excess of the indicated unlabeled oligonucleotides: Mo, NS (nonspecific sequence), and NRGN (lanes 3, 4, and 5, respectively). Incubation with T3R alone (lanes 6 and 8) or RXR alone (lane 9) did not result in any retarded band. B, The presence of T3R in the protein-DNA complex was demonstrated by the addition of an anti-T3R antibody to the reaction (lane 11). The asterisk indicates the complex supershifted with the anti-T3R antibody. Addition of antibody alone (lane 13) or unprogrammed reticulocyte lysate (lane 14) did not produce any shifted band.
T₃RE, we measured the time course of dissociation of the preformed complex after adding a homologous oligonucleotide as a competitor (Fig. 4). These experiments were performed in the presence or absence of Mg²⁺ (35). In the absence of Mg²⁺, the complex formed on the Mo T₃RE was very stable, with almost no dissociation after 45 min, a period during which about 20% of the NRGN-receptor complex was dissociated. In the presence of Mg²⁺, the half-life of the Mo complex was about 5 min, and that of the NRGN complex was 2 min.

Role of each T₃RE half site for binding the RXR-T₃R heterodimer

To evaluate the contribution of each NRGN T₃RE half-site to the binding of the RXR-T₃R heterodimer, different mutations in each half-site as well as in the spacer sequence were introduced (Fig. 5A). The oligonucleotides were labeled and incubated with receptors in the presence or absence of the homologous unlabeled oligonucleotide in a molar excess to determine the specificity of the retarded complex (Fig. 5B). The substitution of two thymidines for two guanines in the first half-site (M1) reduced the ability to bind the receptors. The same mutation in the second half-site (M2) completely suppressed the formation of the retarded band. Exchange of the wild-type spacer sequence for another functional sequence, CTTA (M3) (38), produced a similar retarded band as with the NRGN oligonucleotide. In Fig. 5C, the labeled NRGN oligonucleotide was incubated with increasing amounts of competing oligonucleotides, such as the wild-type sequence; the mutants M1, M2, and M3; and a nonspecific sequence (NS). Consistent with the results shown in Fig. 5B, when the wild-type sequence and that with the mutated spacer (M3) were used, a small excess of cold oligonucleotide strongly decreased the labeled DNA-protein complex, whereas a nonspecific sequence had no competitor activity. Modification of the first half-site (M1) decreased the competing effect, but did not obliterate it completely. In contrast, when the second half-site was modified (M2), the competing effect of the oligonucleotide was completely lost. These results suggest that the integrity of the second half-site is essential for binding the RXR-T₃R heterodimer.

Discussion

Control of the RC3 gene might be an important feature of thyroid hormone action in the brain, not only during the critical developmental stages, but also in adult animals (15, 17). RC3 has been shown to be under thyroid control in rats and goats, but it is not known whether the human RC3 homolog, NRGN, is under similar control in humans. Several genes have been reported to be regulated by thyroid hormone in some species but not in others, such as pituitary GH, which is under control by T₃ in rats but not in humans (39). Considering the severe consequences of thyroid hormone deprivation on human brain development and function (40–
42), it is important to identify what genes from those found to be regulated in the rat are also regulated by thyroid hormone in the human. Moreover, the RC3 gene is of great interest because its control by thyroid hormone is reversible, even in adult animals. Therefore, if regulated in humans, it might be responsible for some of the effects of hypothyroidism on mental states, such as impaired ability to learn and memory loss. In this respect, even mild deficiencies of thyroid hormones, such as those present in subclinical hypothyroidism, are often accompanied by impaired memory (43). As RC3/NRGN is involved in postsynaptic mechanisms leading to various forms of synaptic plasticity, it is tempting to postulate that an impaired control of NRGN expression might underlay some of these symptoms.

The presence of a T3RE in the first intron of the gene, as shown here, strongly suggests that NRGN is a target of thyroid hormone in the human brain. The T3RE was localized using a previously described technique that allowed the isolation of T3 target genes from genomic libraries (34, 44). Further characterization of the T3RE sequence was carried out by footprinting, EMSA, and trans-activation analysis. The footprinted sequence from NRGN contains an imperfect DR4 sequence (ggGgATTAAAtgAGGTAA), with the second half-site almost identical to the consensus sequence. It binds the RXR-T3R heterodimer, but not T3R monomers or dimers, as with other DR4 sequences that contain guanosine in the spacer sequence between the half-sites and lack TC or TA motifs upstream of the first half-site (25). The half-life of the complex is similar to that of the strong T3RE present in the Moloney virus terminal repeat, and the protein complex shifted in the EMSA experiments was supershifted with an anti-T3R antibody. Mutations of the two half-sites revealed the requirement for the integrity of the second half-site to bind the complex, in agreement with the known polarity of the RXR-T3R heterodimer binding to DNA. Current models suggest that T3R binds first to the distal half-site, and subsequent binding of RXR increases binding affinity (45, 46).

Interestingly, NRGN T3RE is located in the first intron of the gene, and preliminary data from our laboratory suggest that a T3R-binding site is located in a similar position in the first intron of the rat RC3 gene, in a conserved region of the intron showing a high degree of similarity to the human gene. Although most of the reported T3REs are located in the upstream promoter region of the target genes, T3REs in intronic locations have also been described, such as in the rat GH gene, NCAM, and Pcp2 (39, 47, 48). Among the thyroid hormone-dependent genes in brain only a few of them have been shown to contain T3REs. These include myelin basic protein (49), NCAM (47), tubulin (47), Pcp2 (48), and PGD2 synthase (50). NRGN is the second human brain gene, after PGD2 synthase (51), in which a T3RE has been identified.

Previous studies in the rat have shown that the control exerted by thyroid hormone on RC3 expression is region specific (16). Some neuronal populations were sensitive, whereas others insensitive to the effects produced by either thyroid hormone deprivation or supplementation. The sensitive regions were layer 6 of the cerebral cortex, layers 2–3 of retrosplenial cortex, the lateral caudate, and the granular layer of the hippocampus. The insensitive regions included, among others, the upper layers of cerebral cortex, the pyra...
midal layers of the hippocampus, and the amygdala. The fact that RC3 expression is modulated by thyroid hormone in specific regions suggested that the T3 control was indirect, so that RC3 could be a distal response subsequent to activation of an earlier responsive gene. However, as pointed out above, T3 induces RC3 expression in cultured cells at the transcriptional level and does not require protein synthesis (21). The finding of a T3RE in NRGN provides further support for a direct interaction of thyroid hormone receptors with regulatory sequences in this gene. Therefore, the reasons for differential T3 sensitivity remain to be determined. We have previously discarded the idea that this phenomenon is due to differential expression of thyroid hormone receptor isoforms (36). Other possibilities to be explored are differential expression of T3R coregulators or an effect due to chromatin conformation.

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