*Gene Expression*, Vol. 5, pp. 169-179, 1996


1052-2166/96 $5.00 + .00


# Differential Regulation of Transcription by the NURR1/NUR77 Subfamily of Nuclear Transcription Factors

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NURR1 is an orphan member of the nuclear receptor superfamily of transcription factors that shares close sequence homology to the orphan nuclear receptor and immediate early gene product NUR77(NGF1$\beta$). The physiological role of NURR1 has not been established in mammalian cells. However, the observation that NURR1 and NUR77 interact with at least one common enhancer element (AAAAGGTCA), together with their partly overlapping but differential expression patterns in mammalian tissues, suggests that these proteins may have both shared and independent transcription regulatory functions. To identify potential target genes that may be regulated by NURR1, we analyzed its DNA binding properties to potential *cis*-acting enhancer elements. Using point mutagenesis of the AAAAGGTCA motif, we have identified three additional sequences that bind specifically to both NURR1 and NUR77, one of which serves as a functional enhancer element. Comparative analysis of the transcription regulatory properties of NURR1 and NUR77 indicates that the proteins can display opposing transregulatory activities that are influenced by the specific *cis*-acting sequences to which they bind. Our results indicate that the transcriptional responses of specific target genes to the NURR1/NUR77 subfamily may be differentially regulated by the relative cellular levels of NURR1 and NUR77 and influenced by the specific enhancer sequences that mediate their activity. Finally, we have identified several potential target genes of neuronal and neuroendocrine origin whose promoters contain this element.

Orphan receptor  Transcriptional regulation  Consensus sequence  Target genes

THE nuclear receptor superfamily comprises a group of structurally related transcription factors that program developmental, physiological, and behavioral responses to a variety of chemical signals. The family includes nuclear receptors for steroids, certain vitamins, and thyroid hormone (3,13,45) in addition to a growing number of orphan members whose physiological function and cognate ligand, if any, remain to be established (36). Nuclear receptors regulate the expression of specific target genes by interacting as either monomers (17,51) or dimers (8,16,22,33,38) with specific DNA elements based on extensions or repeated spatial variations of two types of core sequence motifs, AGGTCA and AGAACA (21,30,44,46). DNA sequence-specific binding by nuclear receptors is specified by a highly conserved DNA binding domain that consists of two zinc finger structural motifs and is the hallmark of this transcription factor family (13,15). Binding of nuclear receptors to DNA and regulation of transcription through specific *cis*-acting sequences may occur in a ligand-dependent or -independent manner (2,11,19,28,39).

The structural features of orphan receptors that are common with previously characterized nuclear receptors has yielded important information on their potential roles as transcription factors. In

Received July 28, 1995.
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particular, information on their mechanisms of interaction with enhancer DNA elements has allowed the identification of some orphan receptor-regulated target genes (52) and has facilitated efforts to uncover the physiological pathways controlled by these transcription factors.

We previously reported the isolation of the orphan receptor, NURR1 (also called RNR-1 and NOT) (24,29,43). Constitutive expression of this protein in the adult mouse is observed predominantly in brain tissue (24), suggesting a role for this transcription factor in regulation of gene expression in the CNS. However, analysis of the expression of the rat (RNR-1) and human (NOT) counterparts of NURR1 indicates that the protein also is the product of an immediate early gene whose expression is induced in response to a distinct stimuli in cells that are not associated with the CNS (29,43). These observations indicate that both constitutive and inducible expression of NURR1 contribute to its tissue specific functional roles.

Analysis of the primary structure of NURR1 indicated that it is highly homologous to a previously identified orphan receptor, NUR77 (NGF1$\beta$) (18,31). NUR77(NGFI$\beta$) was originally identified as an immediate early gene product whose expression is induced by growth factors (18,31,48). Induced expression of the protein has since been demonstrated in a variety of cells in response to a variety of extracellular signals, including neurotransmitters (1,47), polypeptide hormones (10,52), and T-cell antigens (26,54). In two of these cases, NUR77(NGFI$\beta$) has been shown to transduce a specific extracellular signal into changes in gene expression. The induction of NUR77 expression in adrenal cortical cells by adrenocorticotrophic hormone (ACTH) has been shown to mediate regulation of steroidogenesis by activating the steroidogenic enzyme, steroid-21$\alpha$-hydroxylase (52). The protein also mediates programmed cell death of self-reactive T cells in response to apoptotic signals (26,54). Like NURR1, NUR77 also is constitutively expressed in certain tissues (24). However, the constitutive expression pattern of NUR77 is much less restricted than NURR1.

An enhancer DNA sequence has been identified that contains a single AAAAGGTCA (NBRE) motif, binds monomeric NUR77(NGF1$\beta$), and can mediate constitutive transactivation of target gene expression by this orphan receptor (9,49). NUR77(NGFI$\beta$) binds to this enhancer as a monomer (49,51) and binding requires the participation of amino acid sequences within and downstream of the DNA binding domain. These include a five amino acid sequence at the base of the first finger of the DNA binding domain (P-box) that specifies recognition of the core DNA sequence motif by nuclear receptors (46) and an additional motif called the A-box, located adjacent to the C-terminal side of the DNA binding domain that is required for contacting two additional A residues upstream of the core motif (50). As predicted by the high degree of homology between NURR1 and NUR77 in the DNA binding domain and particularly the absolute conservation of P- and A-box sequences, NURR1(RNR1) has been shown to bind and transactivate target gene expression through the same enhancer element (43). Further, the protein also is capable of interacting as a heterodimer with sequences containing direct repeats of the enhancer motif (38). These findings suggest that NURR1 and NUR77 may regulate overlapping gene networks by competing for a common response element if expressed in the same cells.

To identify potential target genes for NURR1, we have carried out DNA binding analysis of point mutants of the AAAAGGTCA motif. Here we identify three additional elements that bind both NURR1 and NUR77 in vitro. Using cell-based transactivation assays, we show that at least one of these novel elements serves as a functional enhancer to mediate transactivation by NURR1 and NUR77. Further, we provide evidence that NURR1 and NUR77 display different transcription regulatory activities that are influenced by the specific *cis*-acting sequences to which they bind. Finally, we identify several potential target genes of neuronal and neuroendocrine origin that contain this promoter element.

## MATERIALS AND METHODS

### *Plasmid Construction*

The NURR1 and NURR1t (aa 229–598) cDNAs were cloned into the plasmid pT$_7\beta$-6 (34) at the *Nco* I site of the $\beta$-globin linker and the *Sal* I site of the polylinker. NUR77 cDNA was cloned into the pT$_7\beta$-6 vector at the same *Nco* I site and the *Eco* RI site of the polylinker. This generated pT$_7\beta$-NURR1, pT$_7\beta$-NURR1t, and pT$_7\beta$-NUR77, which drives the expression of these cDNAs under the control of the T$_7$ promoter in vitro. For expression in tissue culture cells, NURR1 and NUR77 cDNAs were ligated to the *Eco* RI site of p91023*B*, and expressed under the control of the adenovirus major late promoter (53). The CAT reporter plasmids were made by ligating double-stranded oligonucle-

otides containing two inverted copies of the RE oligonucleotide (→←) separated by 10 nucleotides, into the *Bam* HI site of $pBL_2CAT$, upstream of the minimal thymidine kinase promoter (27). All constructs were sequenced by the dideoxy method (42).

*In Vitro Transcription and Translation*

In vitro transcription and translation was accomplished with the TNT kit (Promega, Madison, WI) with the addition of RNAsin (Promega). NURR1 and NUR77 were transcribed with $T_7$ RNA polymerase from $pT_7\beta$-6 recombinant plasmid (34), a derivative of pGEM 2 in which the $\beta$-globin insert of $pSP_6$ H$\beta\Delta$6 (23) is inserted at the initiation codon to create the sequence 5′-CCATGCCTCGACCATGG-3′. The translation was carried out in the presence of either [$^{35}$S]methionine and run on an 8.5% denaturing gel or cold methionine for use in the mobility shift assay, according to the manufacturer's directions. Translation mixture (2.5 $\mu$l) was used in each gel shift binding reaction.

*Electrophoretic Mobility Shift Assay*

Electrophoretic mobility shift assays were performed with in vitro-translated proteins in a rabbit reticulocyte lysate system (TNT, Promega). Proteins were mixed with 100,000 cpm of klenow-labeled probes in the reaction buffer, 20 mM HEPES, pH 7.9, 5 mM $MgCl_2$, 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 8% Ficoll, 600 mM KCl, 500 ng/$\mu$l poly(dI-dC), and 50 mM DTT. The reaction was incubated for 20 min at room temperature and then electrophoresed through a 5.5% nondenaturing polyacrylamide gel in 0.5 × Tris-borate-EDTA (TBE) electrophoresis buffer. The sequence of the NBRE oligonucleotide is listed below. For competition studies, the reaction was performed as described with the indicated concentrations of unlabeled probe. For mutational analysis (Fig. 2) each nucleotide position 1–9 was replaced with three alternative nucleotides.

```
                1 2 3 4 5 6 7 8 9
NBRE 5′-GATCTCG AAAAGGTCA CGG
             AGC TTTTCCAGT GCCCTAG-5′
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*Cell Culture and Transfection*

CV1 and SK-N-SH cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin at 100 $\mu$g/ml, and streptomycin at 100 $\mu$g/ml in a humidified atmosphere of 5% $CO_2$ and 95% air. Twenty-four hours before transfection, $2 \times 10^5$ cells were plated in 3-cm dishes in DMEM supplemented with 10% fetal bovine serum and the cells were allowed to attach. The cells were subsequently washed with Hank's Balanced Salt Solution (HBSS) lacking calcium and magnesium and incubated in DMEM supplemented with 1% Nutridoma SR for CV1 cells and 10% fetal bovine serum for SK-N-SH cells. To $1 \times 10^{10}$ d1312 adenovirus particles (7), in a volume of 333 $\mu$l HEPES-buffered saline (HBS), DNA in a volume of 250 $\mu$l HBS was added and incubated at room temperature for 30 min. Poly-L-lysine was added—the amount required was based on the size of the DNA used—and incubated at room temperature for 30 min. The DNA-modified virus–poly-L-lysine complex was added to the cells and incubated for 2 h at 37°C. The virus-containing medium was removed and 3 ml of specific medium added to the cells. The cells were incubated at 37°C for 24 h before harvesting.

*CAT Assay*

Each plate of cells was washed once with PBS, scraped into 1 ml TEN buffer (40 mM Tris, 1 mM EDTA, 150 mM NaCl, pH 8.0), and collected by centrifugation at 13,000 rpm for 30 s. Cells were resuspended in 250 mM Tris-HCl, pH 7.5, and lysed by four freeze/thaw cycles. Protein concentrations were determined by the Bradford assay (5). CAT activity was determined by incubating 2.5 $\mu$g protein with 0.2 $\mu$Ci [$^3$H]chloramphenicol (20 $\mu$Ci/Umol) and 250 $\mu$M butryl-coenzyme A in 100 $\mu$l 250 mM Tris-HCl, pH 7.5, for 3 h at 37°C. Acylated chloramphenicol was extracted using a mixture of 200 $\mu$l 2:1 TMPD and xylene and counted in a scintillation counter (6).

## RESULTS

*Monomeric Binding of NURR1 to the AAAAGGTCA DNA Sequence Motif*

Electrophoretic mobility shift analysis (EMSA) was used to determine the ability of NURR1 to bind to the same AAAAGGTCA (NBRE) DNA sequence motif as NGFI$\beta$(NUR77) (Fig. 1). A double-stranded oligonucleotide containing the NBRE element was radiolabeled using $\alpha^{32}$P. Both full-length NURR1 and truncated NURR1-(NURR1t), which lacks the first 229 amino acids, were transcribed and translated in vitro using a rabbit reticulolysate system and incubated with

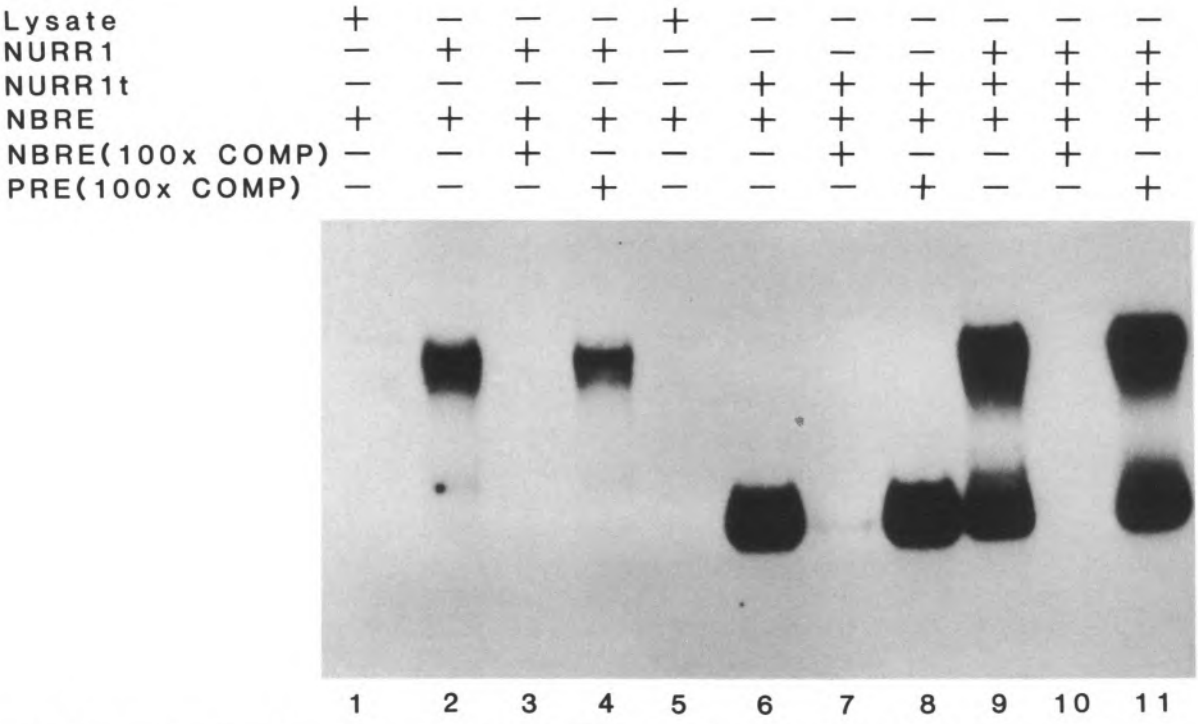


FIG. 1. Electrophoretic mobility shift analysis of NURR1 binding to the NBRE (AAAAGGTCA) DNA sequence motif. Full-length NURR1 and amino-terminal truncated NURR1 (NURR1t; aa 229–599) were transcribed/translated in vitro, incubated separately (lanes 2 and 6) or together (lane 9), with $\alpha^{32}$P-labeled NBRE, and electrophoresed in a 5.5% nondenaturing acrylamide gel. For competition analysis 100 times molar excess of homologous oligonucleotide (lanes 3, 7, and 10) and 100 times molar excess of heterologous oligonucleotide (lanes 4, 8, and 11) were used.

the $^{32}$P-labeled NBRE element. NURR1 and NURR1t bind to the radiolabeled oligonucleotide (Fig. 1, lanes 2 and 6). Binding was successfully competed using 100-fold molar excess of unlabeled homologous oligonucleotide (lanes 3 and 7). No competition was seen using 100-fold molar excess of unlabeled heterologous oligonucleotide PRE (lanes 4, 8, and 11). When NURR1 and NURR1t were cotranslated in vitro, only two radiolabeled DNA–protein complexes were observed corresponding to NURR1–DNA and NURR1t–DNA complexes. The lack of appearance of an additional intermediate band corresponding to a heterodimeric interaction between NURR1 and NURR1t is consistent with the conclusion that, like NUR77/NGFI$\beta$, NURR1 binds to the NBRE as a monomer rather than a dimer.

*Identification of Novel Binding Sites for NURR1*

To identify additional DNA binding sites for NURR1, we introduced point mutations at each position of the AAAAGGTCA element. Nine oligonucleotide pools were synthesized (M1–M9) in which each nucleotide position of the motif was replaced with an equimolar mixture of the three alternate nucleotides. Each oligonucleotide pool was used as a probe in EMSA with in vitro-transcribed and translated NURR1. The results are shown in Fig. 2. NURR1 recognized the oligonucleotide with a G, C, or T at position 1 and the NBRE with approximately equal affinity. Replacement of the A residue at positions 2 and 9 also permitted binding to NURR1, but binding appeared much weaker than observed with the NBRE or M1 DNA elements. Replacement of residues between positions 3 and 8 resulted in loss of binding, suggesting these nucleotides are important for protein binding. Because the M2 and M9 oligonucleotide pools contained a mixture of G, C, and T nucleotides, we investigated whether NURR1 could bind to each of these sequences by generating six additional DNA sequences in which positions 2 or 9 were mutated with either a G, T, or C residue. The results from the binding studies are summarized in Fig. 3. NURR1 bound specifically to the oligonucleotides containing a substitution of a G for an A residue at positions 2 and 9 (Fig. 3A, lanes 5 and 8), but did not bind to the oligonucleotide containing a C or T residue at po-

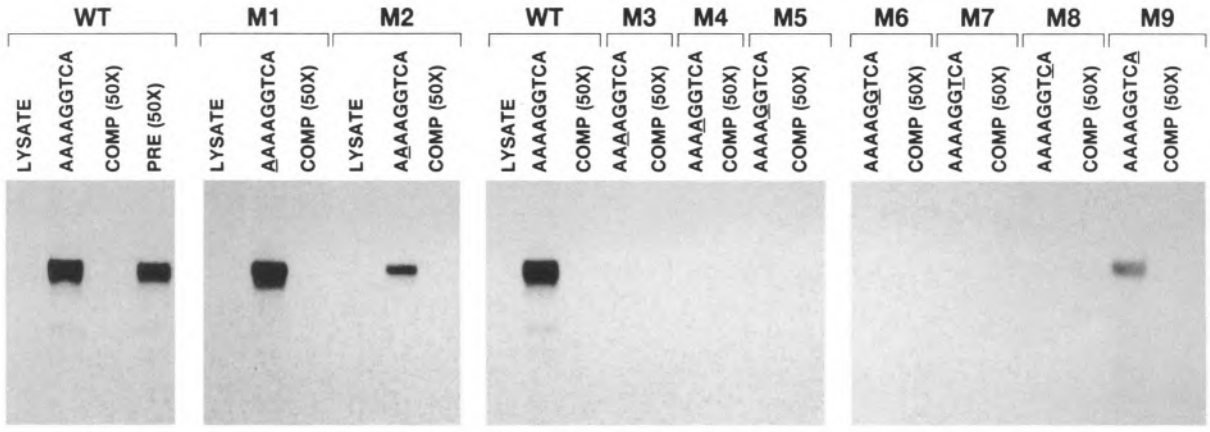


FIG. 2. The relative importance of specific nucleotide bases within the NBRE element (AAAAGGTCA) was examined. Single position nucleotide substitutions in the AAAAGGTCA motif were introduced by replacing the specific nucleotides at each position with a mix of the three alternative oligonucleotides (M1–M9). The series of mutant oligonucleotides were tested for their ability to specifically bind to NURR1 by EMSA.

sition 2 or 9 (data not shown). NURR1 also bound specifically to a combination oligonucleotide M2M9G, containing a G residue at position 2 and 9 (Fig. 3A, lane 10).

To establish whether NUR77 exhibited the same sequence preference for binding to the novel DNA binding sites as NURR1, we examined the binding of in vitro-translated NUR77 to the NURR1 binding sites. The results of this EMSA analysis are shown in Fig. 3B. NUR77 also bound specifically to the same three mutated DNA sites in addition to the NBRE, suggesting that both proteins have similar sequence binding preference.

### *Relative Binding Affinities of NURR1 and NUR77 to the NBRE, M2G, M9G, and M2M9G Consensus Sequences*

To determine the relative binding affinities of NURR1 and NUR77 for the four binding sites identified, in vitro-translated NURR1 or NUR77 was incubated with the radiolabeled NBRE oligonucleotide. The protein/NBRE mixture was incubated with increasing concentrations (5–100 times excess) of unlabeled double-stranded oligonucleotide containing either the NBRE, M2G, M9G, or M2M9G sequences (Fig. 4). The results in Fig. 4 show that binding of the NBRE to both NURR1 (Fig. 4A) and NUR77 (Fig. 4B) was competed most effectively by the homologous NBRE and M2 sequences. Binding was also inhibited, albeit with lower efficiency, by the M9 and M2M9 sequences. Thus, both NURR1 and NUR77 bind to each of the DNA sequences with the same sequence preference (NBRE $\geq$ M2G $\geq$ M9G $\geq$ M2M9G).

### *Functional Analysis of DNA Binding Sites for NURR1 and NUR77*

To determine whether the novel DNA binding sites serve as functional enhancer sequences, we examined the ability of NURR1 and NUR77 to mediate transcriptional activation through the four DNA elements. A monkey kidney cell line, CV1, and a neuroblastoma cell line, SK-N-SH, were transiently transfected with a reporter construct containing two copies of each element linked to the thymidine kinase promoter and CAT reporter gene (tk-CAT). The results are shown in Fig. 5A (CV1 cells) and Fig. 5B (SK-N-SH cells). Both NURR1 and NUR77 constitutively activate the NBRE-tk-CAT reporter in CV1 and SK-N-SH cells, implying that transactivation of this reporter construct by both proteins is not tissue specific.

NURR1 also activated transcription through the M2G element, but surprisingly, NUR77 showed no significant transcriptional activity through this enhancer element in either cell type. Finally, both NURR1 and NUR77 failed to activate transcription of the reporter gene containing two copies (Fig. 5) or four copies (data not shown) of the M9G or M2M9G sequences.

### *Differential Regulation of Transcription by NURR1 and NUR77*

To further examine the differential transactivation responses to NURR1 and NUR77 using the M2G enhancer sequence, we examined the dose dependency of regulation of reporter gene expression by NURR1 and NUR77. In these experiments, we cotransfected a range of NURR1 or

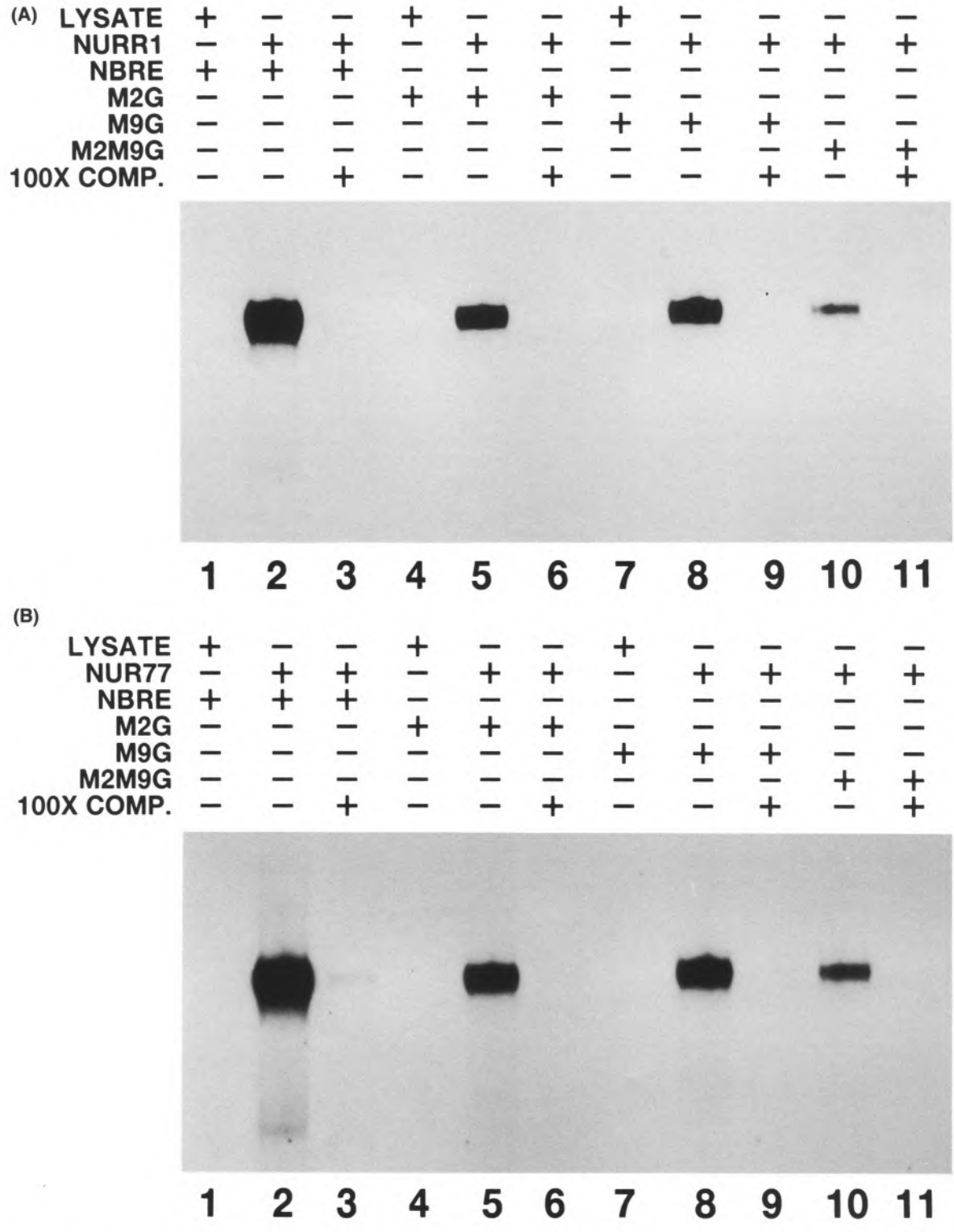


FIG. 3. NURR1 and NUR77 bind to the NBRE, M2G, M9G, and M2M9G sequence motifs. (A) NURR1 and (B) NUR77 were synthesized in vitro, incubated with individual radiolabeled oligonucleotides, and electrophoresed through a 5.5% nondenaturing polyacrylamide gel. Lanes 1, 4, and 7: mock reticulolysate incubated with NBRE, M2G, and M9G, respectfully. Lanes 2, 5, 8, and 10: $^{32}$P-labeled NBRE, M2G, M9G, and M2M9G element incubated with lysate containing NURR1. Lanes 3, 6, 9, and 11: competition analysis with 100 times excess cold NBRE, M2G, M9G, and M2M9G.

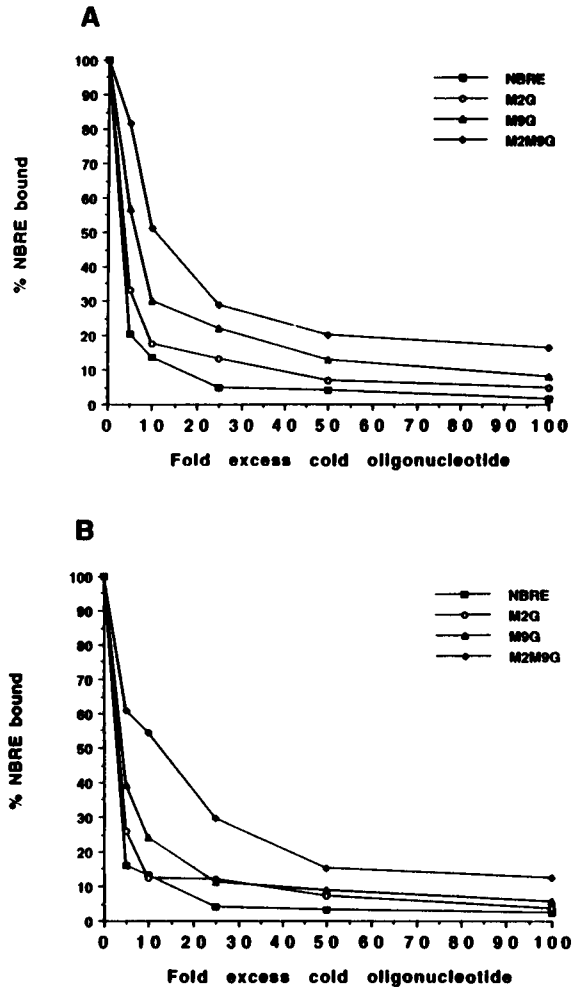


FIG. 4. Relative binding affinities of NURR1 and NUR77 to the NBRE, M2G, M9G, and M2M9G consensus sequences. (A) NURR1 and (B) NUR77 were incubated with radiolabeled NBRE and the retarded complex was competed using increasing concentrations (5–100 times excess) of double-stranded oligonucleotide containing the sequences NBRE-AAAAGGTCA, M2G-AGAAGGTCA, M9G-AAAAGGTCG, and M2M9G-AGAAGGTCG. The protein-DNA complexes were detected by autoradiography, excised from the gel, and counted directly in a scintillation counter. The percent [$^{32}$P]NBRE bound was calculated and plotted.

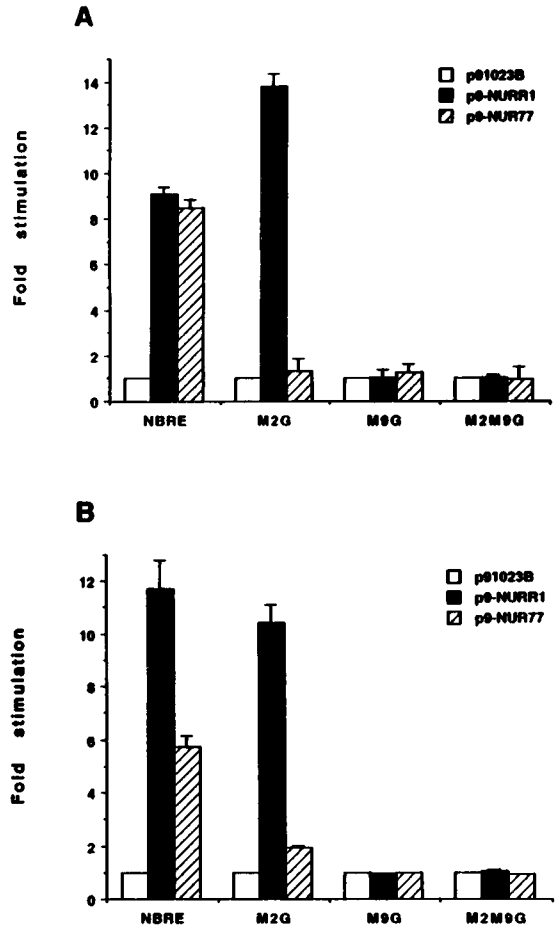


FIG. 5. NURR1 transactivates a reporter plasmid driven by NBRE and M2G DNA sequences. Target plasmid TK-CAT (500 ng), containing two copies of the NBRE (lane 1), M2G (lane 2), M9G (lane 3), or M2M9G (lane 4) sequence motifs, together with 100 ng of either p91023*B* expression vector, p91023*B*-NURR1, or p91023*B*-NUR77 were cotransfected using CV1 cells (A) and SK-N-SH cells (B). Each value is an average of six assays. The fold stimulation is expressed relative to the vector-reporter combination such that a fold induction of one signifies no increase over background.

NUR77 expression plasmid concentrations (12.5–200 ng) together with a constant amount (500 ng) of M2G reporter plasmid into both CV1 and SK-N-SH cells. The results are shown in Fig. 6. NURR1 transactivation of the reporter gene increased in a dose-dependent manner and appeared to saturate at higher doses of NURR1 expression plasmid. In contrast, however, NUR77 activated expression of the gene in both cell types only at low concentrations of the receptor expression plasmid. As the concentrations of NUR77 increased, expression of the reporter gene decreased, with no significant transactivation observed at higher NUR77 expression plasmid concentrations tested.

To establish whether the differential regulation of reporter gene expression by NURR1 and NUR77 was specific for the M2G enhancer element, we carried out similar analyses using the wild-type NBRE-tk-CAT reporter plasmid in both cell lines (Fig. 7). NURR1 and NUR77 showed a similar general pattern of transcriptional regulation of NBRE-tk-CAT to that observed with the M2G-tk-CAT reporter plasmid. The level of transactivation of NBRE-tk-CAT decreased with increasing concentrations of NUR77. However, significant transactivation of this reporter plasmid

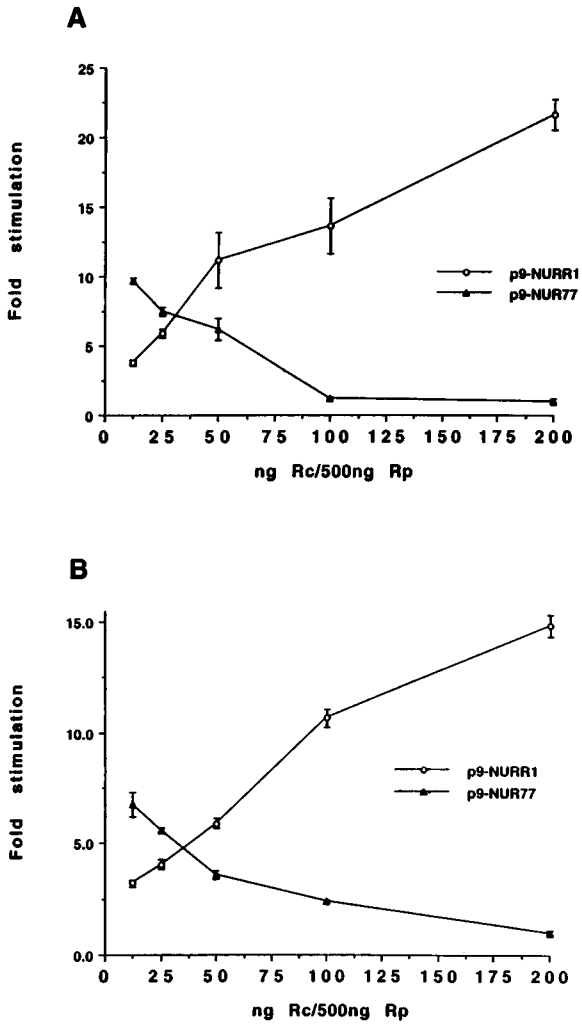


FIG. 6. Comparative analysis of transcriptional regulation by NURR1 and NUR77 through the M2G sequence in CV1 cells (A) and SK-N-SH cells (B). M2G-tkCAT reporter plasmid (500 ng) transfected with increasing concentrations (12.5–200 ng) of NURR1 expression plasmid or increasing concentrations (12.5–200 ng) of NUR77 expression plasmid.

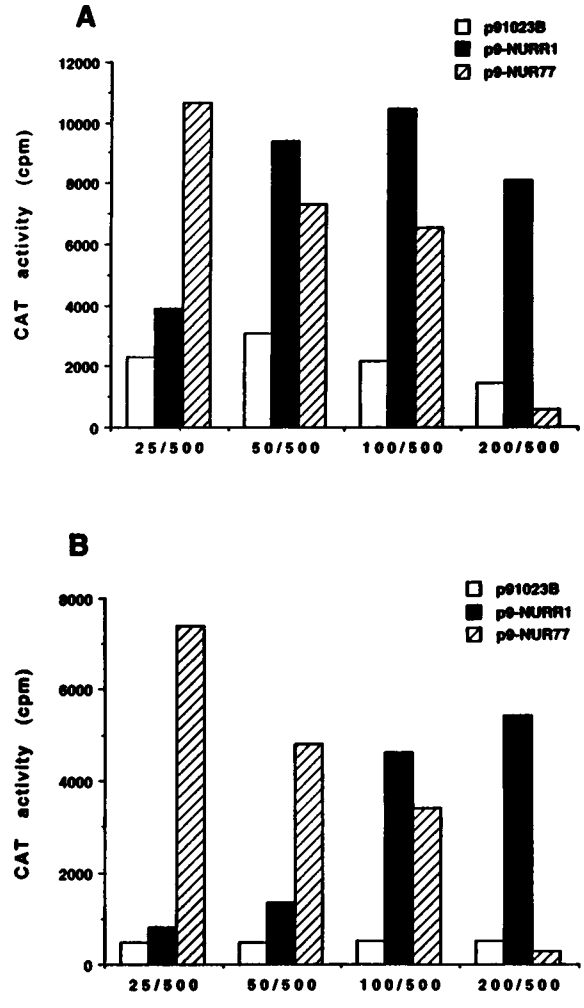


FIG. 7. Comparative analysis of transcriptional regulation by NURR1 and NUR77 through the NBRE in CV1 (A) and SK-N-SH cells (B). NBRE-tkCAT reporter plasmid (500 ng) transfected with increasing concentrations (25–200 ng) of NURR1 expression plasmid or increasing concentrations (25–200 ng) of NUR77 expression plasmid.

was observed at most concentrations of NUR77 tested, indicating that the NBRE-tk-CAT target plasmid is less sensitive to the inhibitory activity of NUR77 in both cell lines tested.

### *Overexpression of NUR77 Can Suppress Transactivation by NURR1*

The decrease in transactivation of the M2G target plasmid using increasing concentrations of NUR77 suggested that overexpression of NUR77 may result in inhibition of transactivation by NURR1 if expressed in the same cells. To test this hypothesis, SK-N-SH cells were cotransfected with M2G-tk-CAT (200 ng), NURR1 expression vector (50 ng), and increasing amounts of NUR77 expression vector (50–100 ng). The results shown in Fig. 8 demonstrate that cotransfection of NURR1 with equal amounts or a twofold excess of NUR77 expression plasmid led to a significant suppression of transactivation of the target gene by NURR1. Thus, NURR1 and NUR77 can display opposing transregulatory activities on the same enhancer element.

## DISCUSSION

In the present study, we have identified three novel *cis*-acting sequences in addition to the previously characterized NBRE site that bind specifi-

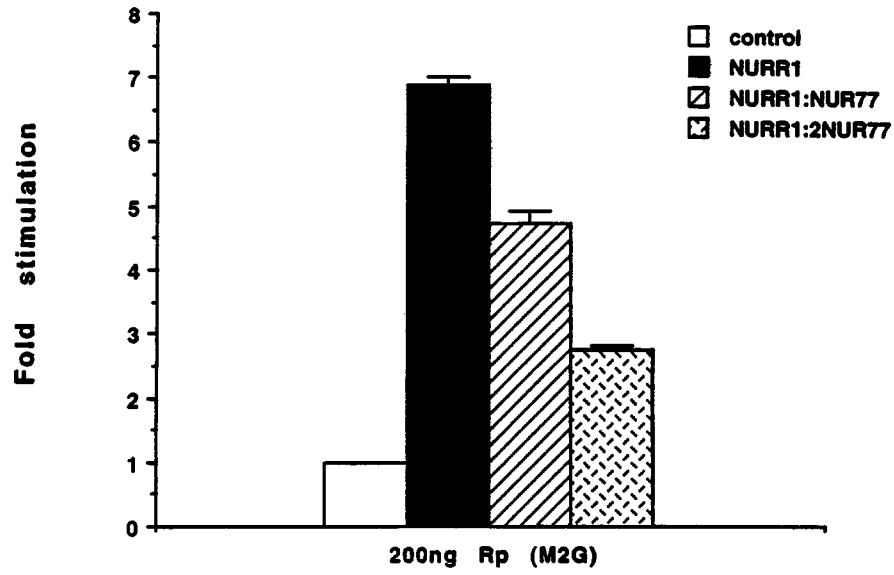


FIG. 8. NUR77 suppresses the transcriptional activation properties of NURR1. Transfection of 200 ng M2G-tkCAT plasmid along with 50 ng p91023*B*-NURR1 and 50 ng p91023*B*-NUR77, or 50 ng p91023*B*-NURR1 and 100 ng p91023*B*-NUR77. The concentration of DNA was kept constant with the appropriate addition of p91023*B* plasmid.

cally to both NURR1 and NUR77. We have demonstrated that at least one of these novel sequences (M2G) serves as a functional enhancer DNA site that mediates transcriptional activation by the NURR1/NUR77 subfamily. Comparative analysis of the transcription regulatory properties of NURR1 and NUR77 using this element indicates that the proteins, at concentrations tested, display opposing transregulatory activities through the same *cis*-acting DNA sequence. The differential transcription regulatory properties of NURR1 and NUR77 are not cell specific but are influenced by the specific enhancer sequences to which they bind. The results indicate that the proteins may differentially regulate the same target genes and that the transcriptional responses of specific target genes to the NURR1/NUR77 subfamily may be differentially regulated by the relative cellular levels of NURR1 and NUR77 and the specific enhancer sequences that mediate their activity.

The observation that NURR1 and NUR77 bind all four DNA binding sites with similar relative affinities is consistent with the observation that the P- and A-box sequences previously shown to participate in DNA binding are conserved between both proteins (24,43,46,50). Analysis of the transregulation of reporter genes containing the potential *cis*-acting sequences by NURR1 and NUR77 demonstrates that only the M2G sequence serves as a functional enhancer element under our experimental conditions. Although it is tempting to speculate that the M9G and M2M9G sequences may function in vivo as negative regulatory elements, the binding of NURR1 and NUR77 to these elements in vivo remains to be demonstrated.

Comparative analysis of the regulation of transcription of the M2G-containing target plasmid by NURR1 and NUR77 reveals that the M2G sequence mediates different and opposing regulatory activities of these proteins in at least two cell types. Whereas NURR1 displays potent, dose-dependent transactivation of the target gene, NUR77 is mostly inactive and is capable of suppressing transactivation by NURR1. The concept of opposing transregulatory activities within nuclear receptor subfamilies interacting with the same enhancer DNA element is not unique to the NURR1/NUR77 subfamily. Previous studies by others have shown that members of the ROR/Rev-erbA nuclear receptor subfamilies can compete for interaction with a common response element to exert opposing transcriptional responses (14,40). In the case of NURR1 and NUR77, our data indicate that the negative regulatory activity of NUR77 is dose dependent. At very low concentrations of NUR77, we observe transactivation of the M2G target plasmid that diminishes rapidly as the concentration of NUR77 increases. These data suggest that selective binding of NUR77 to the

TABLE 1
IDENTIFICATION OF POTENTIAL TARGET GENES

| Consensus Sequence | Potential Target Gene | Position |
|---|---|---|
| M2 AGAAGGTCA | γ mNGF | −854 bp TATA |
| M2 TGACCTTCC | oCRF | −144bp distal TATA |
| M2 CGAAGGTCA | hCRF-BP | −174 bp distal TATA |
| M2 GGAAGGTCA | rPOMC | −31 bp TATA |
| M2 GGAAGGTCA | mPOMC | −31 bp TATA |
| M2 GGAAGGTCA | hPOMC | −34 bp TATA |

Genes of neuronal and neuroendocrine origin whose proximal promoters contain the M2G sequence. γ mNGF, gamma mouse nerve growth factor (12); CRF, corticotropin releasing factor (41); CRF-BP, corticotropin releasing factor binding protein (4); and POMC, proopiomelanocortin (20,32,35).

M2G sequence may facilitate a concentration-dependent removal of essential coactivator proteins or the formation of negative regulatory complexes with this nuclear receptor.

The differential regulation of transcription by NURR1 and NUR77 also is observed when the M2G sequence is replaced by the NBRE enhancer element. However, this sequence is much less sensitive to negative regulation by NUR77 and is transactivated by the protein at most concentrations of NUR77 tested. These data suggest that the dose-dependent differential regulatory activities of NURR1 and NUR77 are also influenced by the specific *cis*-acting sequences to which they bind. DNA sequence-specific-induced conformational changes have previously been shown to play an important role in facilitating both positive and negative transcription regulatory activities of glucocorticoid receptors (25), and it is likely that similar mechanisms underlie the differential enhancer dependent transactivation responses to NUR77.

Examination of GenBank for sequences containing the M2G enhancer element exclusively reveals several genes of neuronal and neuroendocrine origin whose proximal promoters contain this *cis*-acting sequence (Table 1). Preliminary analysis of the spatial expression of NURR1 within the CNS indicates that the protein is expressed in hypothalamic and pituitary structures that express the corticotrophin releasing factor and proopiomelanocortin genes (Saucedo and Conneely, manuscript in preparation). These observations, together with the previous demonstration that the induction of NUR77 in adrenal cortical cells by adrenocorticotropin plays a role in mediating steroidogenesis by regulating the expression of steroid-21-hydroxylase (52), suggest that the NURR1/NUR77 subfamily of nuclear receptors may play an important role in coordinating neuroendocrine regulation of the hypothalamic-pituitary-adrenal axis. Further studies on the regulation of these potential target genes by the NURR1/NUR77 subfamily are currently underway to confirm this hypothesis.

## ACKNOWLEDGEMENTS

The authors would like to thank Aileen Ward for technical assistance. We are very grateful to Dr. J. P. Lydon for critical reading of the manuscript and for the excellent secretarial assistance of Irene A. Harrison.

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