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# The neuron-restrictive silencer element: A dual enhancer/silencer crucial for patterned expression of a nicotinic receptor gene in the brain

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*Contributed by Jean-Pierre Changeux, March 24, 1997*

**ABSTRACT The neuron-restrictive silencer element (NRSE) has been identified in several neuronal genes and confers neuron specificity by silencing transcription in nonneuronal cells. NRSE is present in the promoter of the neuronal nicotinic acetylcholine receptor β2-subunit gene that determines its neuron-specific expression in the nervous system. Using transgenic mice, we show that NRSE may either silence or enhance transcription depending on the cellular context within the nervous system. *In vitro* in neuronal cells, NRSE activates transcription of synthetic promoters when located downstream in the 5′ untranslated region, or at less than 50 bp upstream from the TATA box, but switches to a silencer when located further upstream. In contrast, in nonneuronal cells NRSE always functions as a silencer. Antisense RNA inhibition shows that the NRSE-binding protein REST contributes to the activation of transcription in neuronal cells.**

The mechanisms that account for transcriptional regulation in neurons are still poorly understood. Yet, increasing numbers of transcription factors (1–3) and DNA elements (4, 5) involved in neuron-specific transcription are now characterized. One of them, the neuron-restrictive silencer element (NRSE; ref. 6), also called RE (7), behaves as a regulatory sequence of several neuronal genes (8) by silencing transcription in nonneuronal cells (6, 7, 9–14). The silencing activity of NRSE was primarily studied by transient transfection assays in the promoter of the genes encoding type II sodium channel (NaII; 7) and SCG10 protein (6). It was also recognized in the genes encoding synapsin I (9), nicotinic acetylcholine receptor (nAChR) β2-subunit (5), Ng-CAM (11), m4 muscarinic receptor (12, 13), and choline acetyltransferase (ChAT; 14). On the basis of sequence homologies, Schoenherr *et al.* (8) recently identified about 20 genes carrying a NRSE-like sequence. Among these genes, 17 are expressed in the nervous system and in 10 of them, including the nAChR β2-subunit gene (5), NRSE is located in the 5′ untranslated region (UTR) (ref. 15; see also refs. 16 and 17). Although important functional elements have already been found in the 5′ UTR or in intragenic positions (4, 18–20), NRSE is, to our knowledge, the first element sharing a conserved intragenic position in several genes. The functional significance of such an unusual position remained to be explained.

A transcription factor of the Gli-Krüppel zinc-finger family that binds to NRSE has recently been characterized in HeLa cells, and named neuron-restrictive silencer factor (NRSF; ref. 15) or RE1 silencing transcription factor (REST; ref. 21, see also ref. 22). *In vitro* studies revealed that REST exhibits a transcriptional repressing activity on promoters that carry the NRSE sequence (15, 21, 23).

In this work, we have analyzed the function of NRSE in the promoter of the mouse gene encoding the nAChR β2-subunit as well as the importance of its location within the promoter. In transgenic mice, we show that upon mutation of NRSE, the pattern of expression of the β2-subunit gene promoter dramatically changes but remains restricted to the nervous system. We confirm that NRSE *in vitro* can behave either as an enhancer or as a silencer depending both on the primary structure of the promoter and the type of cell line. We further show by antisense experiments that in neurons, REST contributes to the activation of transcription via NRSE. A model for the regulation of transcription by REST/NRSE in neurons is proposed.

## MATERIALS AND METHODS

**Plasmids.** NRSE-SVP, an oligonucleotide containing three NRSE sequences of the β2-subunit gene, was inserted into the *Not*I/*Pst*I sites of SVP-Luci (described in ref. 4). For NRSE-TATA and TATA-Luci, NRSE-SVP and SVP-Luci were restricted by *Sal*I/*Nco*I filled with the klenow enzyme. The largest restriction fragment was self-ligated. TATAIIB, the small *Pst*I/*Xba*I fragment of 40IIB-MyHC-CAT (ref. 24; kindly provided by Thierry Diagana, Institut Pasteur, Paris) was inserted into SVP-Luci. The NRSE oligonucleotide was then inserted into the *Not*I/*Pst*I sites to obtain NRSE-TATAIIB. The plasmids mutated in NRSE were constructed using a mutated oligonucleotide (NRSE-Mut). NRSE-spacer-TATA, the 198-bp *Pst*I/*Bsp*HI fragment from the rat GluR1 cDNA (ref. 25; accession number X17184) was inserted into the *Pst*I/*Nco*I site of NRSE-SVP. The different lengths of spacers were obtained by PCR. uTATA-Luci, the *Bgl*I/*Sal*I fragment of TATA-Luci, was excised and the plasmid was self-ligated after action of the T4 DNA polymerase. TATA-272-NRSE, the *Bgl*II/*Hin*dIII fragment of NRSE-SVP, was inserted into the *Stu*I/*Hin*dIII sites of uTATA-Luci. uTATA-272-Luci, the NRSE sequence, was excised from TATA-272-NRSE by *Sac*II/*Pst*I. This plasmid was used to normalize the transcriptional activity of TATA-272-NRSE. TATA-34-NRSE, the *Sfi*I/*Sac*II fragment of TATA-272-NRSE plasmid, was excised. The transcriptional activity of this plasmid was normalized to that of uTATA-Luci. The plasmids were sequenced to rule out mistakes of the *Taq* DNA polymerase. CMV-TSER, the 4100-bp *Eco*RI fragment of the REST-EXPRESS plasmid (ref. 21; kindly provided by Gail Mandel, University of New York, Stony Brook), was inserted into the

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Abbreviations: nAChR, nicotinic acetylcholine receptor; NRSE, neuron-restrictive silencer element; β-gal, β-galactosidase; 5′ UTR, 5′ untranslated region; REST, RE1 silencing transcription factor; CNS, central nervous system.

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appropriate sites of pBluescript (Stratagene) and then in the *Hin*dIII/*Xba*I site of pcDNA IAmp (Invitrogen).

**Transgenic Mice and Immunohistochemistry.** The luciferase gene from luciferase plasmids was excised and replaced by the nlsLacZ gene. The $\beta$2-promoter/nls*LacZ* fragment was injected into fertilized oocytes of FVB or B6SJL mice. Staining of tissues was performed as described (5). The integration of the transgene was tested by PCR using DNA extracted from the tail or the yolk sac. Immunohistochemistry was performed on transgenic mice T9 (carrying the mutated promoter), which is described in Table 1. Mice were perfused with 4% paraformaldehyde, the brain was extracted and cut (50-$\mu$m sections) using a vibratome (Leitz). The slices were incubated overnight at 4°C in an anti-GFAP serum (Dako), followed by a biotynilated anti-rabbit antibody solution, and finally revealed by a peroxydase–streptavidin complex.

**Gel-Shift Experiments.** Nuclear extracts were prepared from $\approx 10^7$ cells as described (5). For binding, 1 nmol of labeled oligonucleotide was mixed with 0.5 $\mu$g of protein extract in 10 mM Hepes, pH 8/10% glycerol/0.1 mM EDTA/0.1 M NaCl/2 mM DTT/0.1 mg/ml BSA/4 mM $MgCl_2$/4 mM spermidine/1 mM phenylmethylsulfonyl fluoride/1 $\mu$g polydIdC in 20 $\mu$l. The reaction was incubated for 10 min on ice. The DNA–protein complexes were then analyzed on a 5% polyacrylamide gel.

**Reverse Transcription–PCR (RT-PCR).** The mRNA (4 $\mu$g) was hybridized for 5 min at 80°C with 50 pmol of poly dT. The synthesis of the cDNA was performed using 400 units of MMLV reverse transcriptase (Gibco) for 45 min at 37°C in the buffer recommended by the supplier. One-tenth of the reverse transcription was amplified using Promega's *Taq* DNA polymerase (30 cycles, 94°C; 1 min, 55°C; 45 sec, 72°C; 1 min) in the buffer recommended by the supplier in 2.5 mM $MgCl_2$ using the primers 5′-GAATCTGAAGA(A/G)CAGTTTGT-GCAT and 5′-TTTGAAGTTGCTTCTATCTGCTGT. When detecting mRNA from neuroblastomas, we performed a second PCR amplification using one-tenth of the first amplification using the following primers: 5′-GAAGA(A/G)CAGTT-TGTGCATCACATC and 5′-GTTGCTTCTATCTGCT-GTTTTGTA.

**Cells and Transfection.** Neuroblastomas SK-N-Be and 3T6 fibroblasts were grown in DMEM + 10% fetal calf serum (FCS) supplemented with 1% glutamine and 1% streptomycin. PC12 cells were grown in the same medium + 10% FCS. For transfection, cells were plated at 1 to $2.10^5$ cells per ml in 35-mm dishes and transfected with 1 $\mu$g of DNA using calcium phosphate the next day. The luciferase activity was measured 48 hr later. When plasmid activities were compared, all plasmids were prepared the same day. At least two different DNA preparations were tested for each plasmid. All transfections were done in triplicate and repeated at least four times.

**Oligonucleotides.** The following oligonucleotides were used: NRSE, 5′-GGCCC(TTCAGCACCACGGACAGCGCT-C)$_3$TGCA; NRSEMut, 5′-GGCCC(TTCAGCACCACTTAC-AGCGCTC)$_3$TGCA; and SP1, 5′-TCGACTAATCTCCGCC-CAGTTC.

## RESULTS

***In Vivo* Demonstration of the Silencer/Enhancer Function of NRSE from the nAChR $\beta$2-Subunit Gene Promoter.** To understand the role of NRSE in neuronal gene expression *in vivo*, transgenic mice were constructed with the 1.2-kbp transcription control sequences of the $\beta$2-subunit gene containing

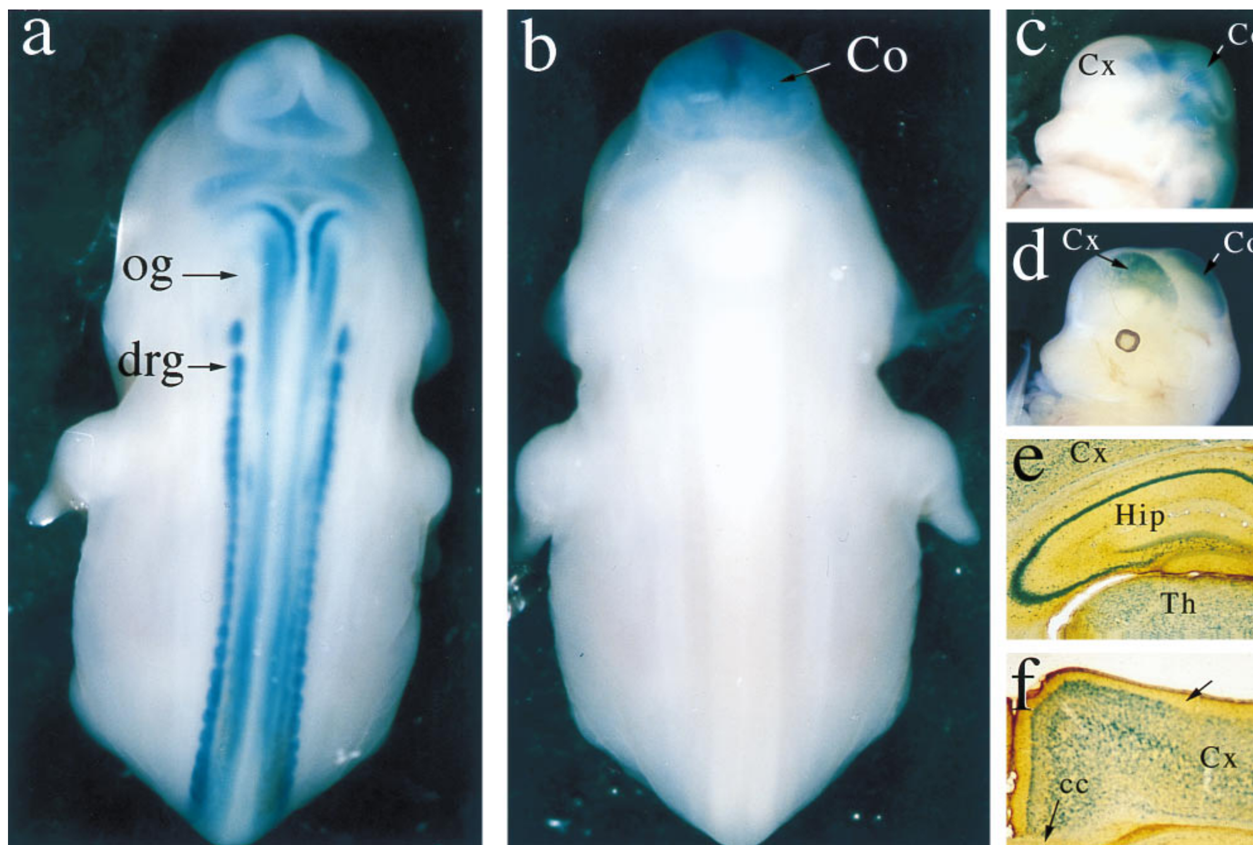


FIG. 1. Point mutation of NRSE in the promoter of the nAChR $\beta$2-subunit gene changes the pattern of $\beta$-gal expression in transgenic mice. (*a–d*) Whole-mount coloration of E13.5 transgenic embryos carrying the wild-type promoter (*a* and *c*) and with point mutation in NRSE (*b* and *d*). (*e* and *f*) Detection of the $\beta$-gal activity and the glial fibrillary acidic protein immunoreactivity in the adult mutated transgenic brain. (*e*) Dorsal part of the hippocampus. (*f*) Dorso-medial part of the cortex, showing the absence of $\beta$-gal expression in the molecular layer (arrow). og, orthosympathetic ganglia; drg, dorsal root ganglia; Co, colliculus; Th, thalamus; Cx, cortex; Hip, hippocampus; cc, corpus callosum.

either wild-type or point-mutated NRSE. This mutation was shown to abolish protein binding to NRSE (6). As described previously (5), and as shown in Fig. 1, at embryonic day 13.5 (E13.5) the wild-type promoter determined a widespread expression of the β-galactosidase (β-gal) transgene in the peripheral nervous system and in different structures of the central nervous system (CNS) such as the thalamus, the colliculus, or the metencephalon (Fig. 1 *a* and *c*; Table 1). In the adult CNS, staining was restricted to a subset of neurons in the hypothalamus (5). After NRSE mutation, the pattern of β-gal staining changed dramatically. In nine independent transgenic embryos studied at E13.5, expression of β-gal was switched off in all previously labeled structures except in the colliculus (see Fig. 1 *b* and *d*; Table 1), whereas it was switched on in the cortex. In the adult brain, the mutated promoter drove expression in the vast majority of the CNS neurons (Fig. 1 *e* and *f*). At the cell level, β-gal activity was essentially detected in neurons (Fig. 2 *a* and *b*) and exceptionally in few oligodendrocytes of the white matter (Fig. 2*c*). The β-gal expression could not be consistently detected in other non-neuronal cells or in other organs (Table 1).

**Analysis of the Silencer/Enhancer Function of NRSE Within Synthetic Promoters.** It has been previously reported that NRSE acts as a neural-restrictive element by silencing transcription in nonneuronal cells, but does not exhibit intrinsic regulatory activity in neurons (6–11, 13, 14). We investigated this apparent discrepancy with our *in vivo* studies by transfection analysis. NRSE was first fused upstream from the ubiquitous 300-bp simian virus 40 (SV40) early promoter or from a minimal promoter carrying only a TATA box upstream from the luciferase gene (Fig. 3*A*) and transfected into neuronal (neuroblastomas and PC12 cells) and nonneuronal cell lines (fibroblasts). As expected, NRSE decreased transcription of SV40 promoter in fibroblasts cells (Fig. 3*B*). Unexpectedly, we found that NRSE also behaved as a silencer in neuroblastoma cells (Fig. 3*B*). When linked upstream of a minimal promoter, NRSE still silenced transcription in nonneuronal cells, but surprisingly switched to an enhancer in neuronal SK-N-Be as well as in PC12 cell lines (Fig. 3*B*). A similar activation by NRSE was observed with an unrelated minimal promoter (Fig. 3*B*).

**The Primary Structure of the Promoter Controls the Silencer/Enhancer Switch in Neuroblastomas.** In the reports that suggested the absence of regulatory activity of NRSE in neuronal cells (6–14, 26), NRSE was located between 1,500 and 200 nucleotides upstream from the transcription start site. In the synthetic NRSE-TATA promoters, it is only 50 bp upstream from the TATA box. We thus tested a putative distance dependence of NRSE activity by inserting spacers of variable length between NRSE and the TATA box. Fig. 4*B* shows that in neuroblastoma cells, NRSE enhanced transcription when located at less than 50 bp from the TATA box, but switched to a weak silencer when located further upstream (compare the activities of plasmids with 50 and 100 bp spacers in Fig. 4*B*). In fibroblast cells, NRSE did not repress transcription when abutted to the TATA box but consistently decreased transcription when located further than 20 nucleotides from the TATA box. Interestingly, the intervening sequences between NRSE and TATA are different in NRSE-TATA and in NRSE-50-TATA, but the enhancer/silencer activity is the same. This proves that the regulatory activity is not due to spacer sequences.

In the β2-subunit gene (5) as well as in half of the neuronal genes in which NRSE was detected, this element is located in the 5′ UTR (8). We thus tested the cis regulatory activity of NRSE located in the 5′ UTR using synthetic promoters. Fig. 4*C* shows that in fibroblast cells, when located in the 5′ UTR, NRSE was still a repressor at 272 bp but did not show regulatory activity when abutted to the TATA box. Interestingly, Fig. 4*C* shows that in neuroblastoma cells, NRSE located in the 5′ UTR always behaves as an enhancer, even at more than 50 bp downstream from the TATA box.

**REST mRNA and NRSE Binding Activity Are Present in Both Neurons and Fibroblasts.** We next attempted to identify the protein complexes able to interact with NRSE. Previous works had shown that in fibroblast cells, the only protein that binds to this sequence was REST (15, 21), whereas no NRSE binding activity was observed in neuronal cell lines (6, 7, 13). Yet, in our hands, gel-shift experiments with a probe containing NRSE revealed a single specific complex that migrated at the same position with extracts prepared from both fibroblast or neuroblastoma cell lines (Fig. 5 *Left*). However, the signal was significantly lower in neuroblastomas than in fibroblasts (see Fig. 5 *Left*). The observed complex migrated slowly, as expected for REST (8, 15, 21, 22, 26). The same complex could be observed with an oligonucleotide made up of three NRSEs (not shown). The presence of REST mRNA in both neuroblastoma and fibroblast cells was confirmed by an RT-PCR experiment (Fig. 5 *Right*). Whereas REST mRNA from fibroblast cells was easily detectable, two rounds of PCR amplification using nested oligonucleotides were necessary to detect REST mRNA from neuroblastoma cells (Fig. 5 *Right*) and from PC12 cells (not shown). The REST mRNA and NRSE binding activity are thus present in neurons, probably in lower amounts than in nonneuronal cells (14).

**REST Is Able to Trans-Activate NRSE in Neurons.** Different studies have already shown that overexpression of REST is sufficient to repress the transcriptional activity of NRSE-

Table 1. Anatomical localization of the β-gal-stained cells in transgenic mice constructed with wild-typed (WT) 1,163-bp promoter of the nAChR β2-subunit gene and T1–T10 (same length of promoter point mutated in NRSE).

| Structures | WT | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cortex | — | ± | + | + | + | — | ± | ± | ± | ± | + |
| Superior colliculus | + | ± | + | + | + | + | + | — | + | + | + |
| Thalamus | + | — | ± | — | — | — | — | — | — | — | — |
| Mesencephalon | + | — | — | — | — | — | — | — | — | — | — |
| Hypothalamus | ± | — | — | — | — | — | — | — | — | — | — |
| Basal telencephalon | ± | — | — | — | — | — | — | — | — | — | ± |
| Spinal cord | ± | ± | — | — | — | — | — | — | — | — | — |
| Brain stem | + | — | ± | — | — | — | — | — | — | ± | + |
| Otic ganglion | + | — | — | — | — | — | — | — | — | — | — |
| Eighth ganglion | + | — | — | — | — | — | + | — | — | + | + |
| Superior cervical ganglion | + | ± | — | — | — | — | — | — | — | — | — |
| Dorsal root ganglion | + | ± | — | — | — | — | — | — | — | — | — |
| Trigeminal ganglion | ± | — | — | — | — | — | — | — | — | — | ± |
| Cartilage (vertebra) | — | ± | — | — | — | — | — | — | — | + | — |
| Mouth mucosa | — | — | + | — | — | — | — | — | — | + | + |

Mice T9 and T10 have stably integrated the transgene. —, no staining.

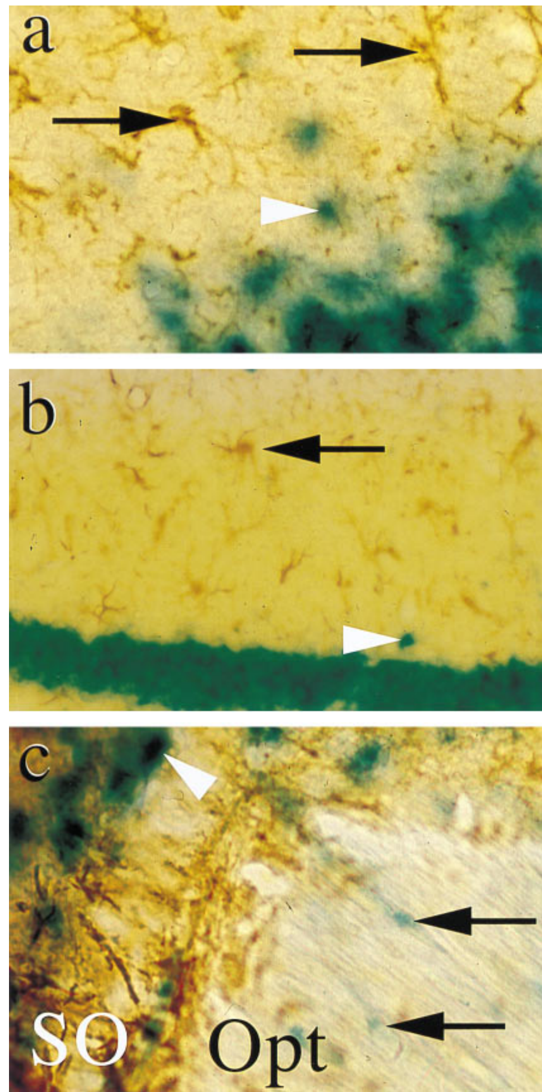


FIG. 2. Expression of the NRSE-mutated nAChR $\beta$2-subunit gene promoter is restricted to neurons. The $\beta$-gal activity (arrowheads) and the glial fibrillary acidic protein immunoreactivity (arrows) were compared in different regions of the adult brain. (*a*) Layer 2 of the pyriform cortex. (*b*) Pyramidal neurons of the hippocampus. (*c*) Oligodendrocytes (arrows) of the optic tract (Opt) and neurons (arrowheads) of the Supra-optic nucleus (SO).

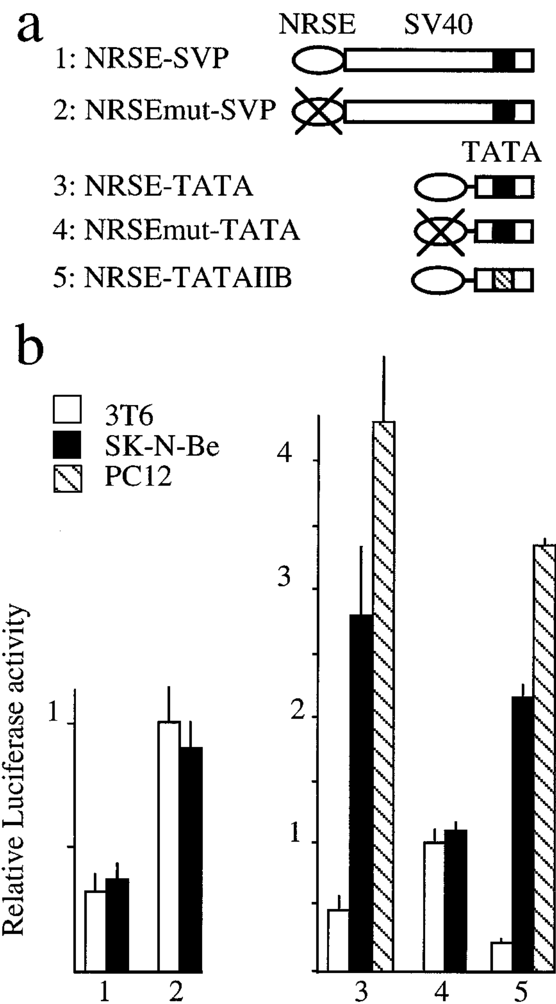


FIG. 3. Enhancer and silencer activities of NRSE within synthetic promoters. (*a*) Schematic representation of the plasmids used for transfection. Oval, three NRSEs; crossed-out oval, mutated NRSEs; solid box, SV40 TATA box; hatched box, myosin IIB TATA box. (*b*) Relative activity of these plasmids transfected in different cell lines. The luciferase activities were normalized to that of the same plasmid containing no NRSE sequences (SVP-Luci, TATA-Luci, or TATAIIB-Luci).

containing promoters (15, 21, 23). To test whether REST by itself is involved in the trans-activation observed in close-located NRSE, neuroblastoma cells were transfected with the synthetic NRSE-TATA promoter together with an expression vector driving the synthesis of a REST antisense mRNA. Fig. 6 shows that upon transfection of REST antisense, the cis-activation of the minimal promoter by NRSE was no longer observed. In contrast, no changes in NRSE cis-activation were detected in control transfection experiments when the same expression plasmid contained no cDNA. This demonstrates that REST trans-activates transcription in neuronal cells when its binding site is located at the proximity of the transcription start site.

## DISCUSSION

NRSE is a regulatory sequence that is present in several neuronal genes (8) and that was, up to now, thought to silence neuronal gene transcription in nonneuronal cells (6, 7, 9–14). We have analyzed the function of NRSE as well as the importance of its position in the promoter of the gene encoding the mouse nAChR $\beta$2-subunit and in synthetic promoters by combining transgenic mice and *in vitro* studies.

Point mutation of NRSE in the $\beta$2-subunit gene promoter studied in transgenic mice dramatically changed the pattern of $\beta$-gal staining (Fig. 1). In fact, at E13.5, expression of $\beta$-gal was, after mutation, switched off in the thalamus, the metencephalon, the dorsal root ganglias, and several other structures, whereas it was switched on in the cortex and remained unchanged in the colliculus (Fig. 1; Table 1). These results demonstrate a new dual-activity of NRSE in neurons as an enhancer or as a silencer depending on the brain area. This silencer activity of NRSE observed *in vivo* in the embryonic cortex or in most of the CNS neurons was confirmed *in vitro* (Fig. 3).

Several reports claimed that in neuronal cell lines, NRSE had no regulatory activity. In fact, these studies were done with the promoters of genes coding for the SCG10 protein (in which NRSE is located at −1472; ref. 6), for the NaII channel (−996; ref. 7), for synapsin I (−231; ref. 9), for Ng-CAM (+1405; ref. 11), for the m4 muscarinic receptor (−574; ref. 13), for the ChAT gene (−1580; ref. 14), and for dopamine $\beta$-hydroxylase (−251; ref. 26). We may explain the apparent discrepancy

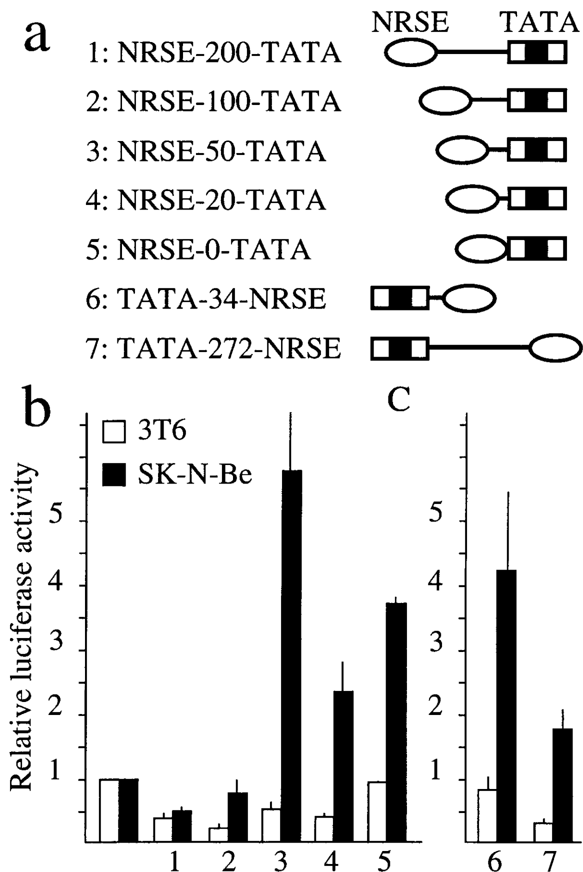


FIG. 4. The sign of the regulatory activity of NRSE depends on the primary structure of the promoter in neuroblastomas but not in fibroblasts. (*a*) Schematic representation of the plasmids. See Fig. 3 legend for explanations of symbols. (*b* and *c*) Relative activities of the plasmids transiently transfected. The activities of the plasmids were normalized to that of the same plasmids with no NRSE.

between these works and ours by the demonstration (Fig. 4) that, in neuronal cells, NRSE actually behaves as an enhancer only when located downstream, or at less than 50 bp upstream from the TATA box, but acts as a silencer when located further upstream, as in the previously described promoters.

NRSE was described as a silencer in nonneuronal cells (6, 7, 9–14, 23). Thus, we anticipated that its mutation would

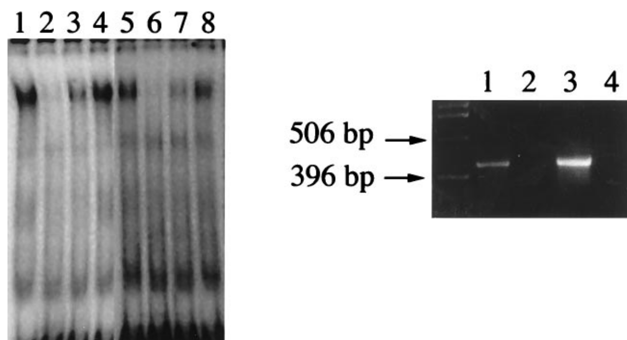


FIG. 5. Detection of NRSE binding activity and REST mRNA in fibroblasts and neuroblastoma. (*Left*) Mobility shift experiment using an NRSE probe and extracts from fibroblast (lanes 1–4) or neuroblastoma cells (lanes 5–8). The gel retardation was done in presence of 100-fold molar excess of cold NRSE (lanes 2 and 6), NRSEmut (lanes 3 and 7), or oligonucleotide SP1 carrying an SP1 site (lanes 4 and 8). (*Right*) RT-PCR with RNA extracted from fibroblasts (lanes 1 and 2, one round of PCR amplification) or neuroblastomas (lanes 3 and 4, two rounds of PCR amplification using nested primers). Lanes 2 and 4, reverse transcriptase was omitted.

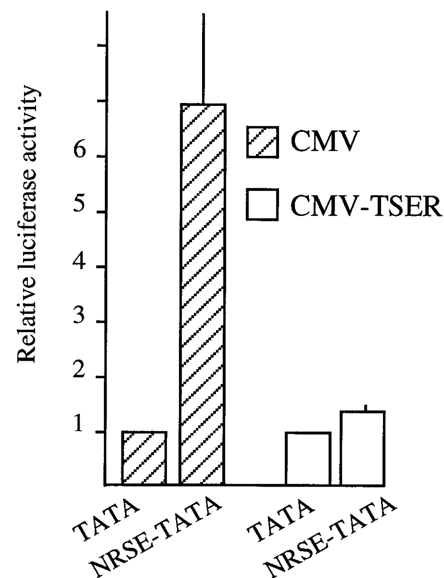


FIG. 6. REST trans-activates transcription through binding to NRSE. Activation of transcription by NRSE was compared after cotransfection with the CMV-TSER plasmid expressing a REST antisense mRNA or with the expression plasmids carrying no cDNA. The activity of the NRSE-TATA plasmid is compared with that of TATA-plasmid cotransfected with the same expression plasmid set to 1.

promote expression of the $\beta$-gal transgene in glial cells and in nonneuronal tissues. Yet, in the nine independent lines of transgenic mice analyzed at E13.5, nonneuronal expression was only rarely observed and not in a reproducible manner (Table 1). Moreover, in the adult brain, comparison of the glial fibrillary acidic protein immunoreactivity and of the $\beta$-gal staining showed that the mutated promoter was primarily expressed in neurons (Fig. 2 *a* and *b*) and exceptionally in few oligodendrocytes of the white matter (Fig. 2*c*). These results confirm that the 1.2-kbp promoter of the $\beta$2-subunit carries other neuron-specific elements (5) that prevent ectopic expression of the NRSE-mutated promoter. When transfected into fibroblast cells, the 1.2-kbp promoter mutated in NRSE was shown to be more active than the wild-type promoter (5). In transgenic mice, the activity of the mutated promoter in nonneuronal cells was probably still below the level of detection.

One possible mechanism that could account for the dual activity of NRSE is that the NRSE-binding factors interact with the general transcription factors (22). At more than 50 bp, no interaction, thus no regulation, would become possible, explaining why NRSE is transcriptionally inactive in distant-NRSE promoters in neuronal cell lines. In fibroblasts cells NRSE behaves always as a repressor except when abutted to the TATA box (as in the VGF gene; ref. 27), in which case no regulatory activity is observed. This could be due to a steric hindrance between the general transcription factors and REST that could not bind to the NRSE. Another possible mechanism would be that the NRSE-binding factors displace repressor factors, yielding increased access of the general transcription factors to the promoter. Such a derepression mechanism appears possible *in vivo*. But it seems less probable in our transient transfection assay, as NRSE would derepress transcription in all cell types, the TATA promoters (IIB and SV40) being relatively equally active in neuroblastoma and in fibroblast cells (not shown).

An NRSE-binding factor (REST) was recently characterized and cloned (15, 21, 22). Using gel-shift experiments and RT-PCR, we have detected the REST protein and mRNA in the fibroblast 3T6 as well as in the neuroblastomas SK-N-Be.

We also detected REST mRNA in the neuronal PC12 cells (not shown). However, in these latter cell lines, the level of mRNA was much lower than in fibroblast cells. The NRSE-binding activity and REST mRNA have consistently been detected in nonneuronal cells (6, 7, 9, 11, 13). In contrast, in neuronal cells results appeared contradictory. For instance, NRSE-binding activity was (26) or was not detected in the neuronal cell line PC12 (6, 7, 9, 13, 23); but the REST protein could be detected upon long exposure of a Western blot made from PC12 proteins (21). In neuroblastomas, the NRSE-binding activity and REST mRNA were (11, 14, 28) or were not (6, 7, 13, 15) detected depending on the studies. Recently, Lonnerberg *et al*. (14) showed unambiguously, by RNase protection assay, that REST mRNA is present but in much lower amounts in neuronal than in fibroblast cell lines. Thus, the apparent discrepancy is probably due to the level of detection of the DNA-binding activity.

The REST protein is a multi-zinc-finger protein with structural homology with the Krüppel class of zinc-finger proteins (8, 21). Our results extend the analogy between REST and Krüppel (Kr) or YY1 beyond the zinc-finger similarity. Actually, when acting through distant binding sites, both Kr and YY1 provide repression almost exclusively (29, 30). When the Kr-binding site is located next to the transcription start site, Kr acts as transcriptional activator when monomeric, whereas Kr dimers, formed at high concentration, cause repression (30, 31). A functional homology thus exists with the phenomenon described in this work, since REST can both activate and repress gene expression depending on the location of its single DNA-binding site. Our results suggest that a probable mechanism of regulation by REST is the following: at low concentration (in neurons) REST does not suffice to repress distant-NRSE promoters (NaII, SCG10, synapsin, etc.), whereas REST enhances transcription of close-NRSE promoters ($\beta$2-subunit, synthetic NRSE-TATA, promoters with 5′ UTR NRSEs). At high concentration [in nonneuronal cells or in neurons transfected with REST expression vectors (15, 21)], REST elicits repression regardless of the promoter context. However, that NRSE is a silencer in the adult neurons of the brain strongly suggests that, in addition to REST, other NRSE-binding proteins are involved in gene regulation.

Other studies have described such dual enhancer/silencer activity of DNA elements (29–32), but to our knowledge, this work is the first demonstration for a role *in vivo* and in the nervous system of higher eukaryotes of such a dual function. It supports the view that the dual activity of REST contributes to the particularly sophisticated regulatory mechanisms that determine the three-dimensional expression of neuronal genes in the brain.

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1. Johnson, J. E., Birren, S. J. & Anderson, D. J. (1990) *Nature (London)* **346,** 858–861.
2. Akazawa, C., Sasai, Y., Nakanishi, S. & Kageyama, R. (1992) *J. Biol. Chem.* **267,** 21879–21885.
3. Lee, J., Hollenberg, S., Snide, R. L., Turner, D., Lipnick, N. & Weintraub, H. (1995) *Science* **268,** 836–844.
4. Bessis, A., Savatier, N., Devillers-Thiery, A., Bejanin, S. & Changeux, J. P. (1993) *Nucleic Acids Res.* **21,** 2185–2192.
5. Bessis, A., Salmon, A., Zoli, M., Le Novere, N., Picciotto, M. & Changeux, J. P. (1995) *Neuroscience* **69,** 807–819.
6. Mori, N., Schoenherr, C., Vandenbergh, D. J. & Anderson, D. J. (1992) *Neuron* **9,** 45–54.
7. Kraner, S. D., Chong, J. A., Tsay, H.-J. & Mandel, G. (1992) *Neuron* **9,** 37–44.
8. Schoenherr, C. J., Paquette, A. J. & Anderson, D. J. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 9881–9886.
9. Li, L. A., Suzuki, T., Mori, N. & Greengard, P. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1460–1464.
10. Pathak, B., Neumann, J., Croyle, M. & Lingrel, J. (1994) *Nucleic Acids Res.* **22,** 4748–4755.
11. Kallunki, P., Jenkinson, S., Edelman, G. M. & Jones, F. S. (1995) *J. Biol. Chem.* **270,** 21291–21298.
12. Mieda, M., Haga, T. & Saffen, D. (1996) *J. Biol. Chem.* **271,** 5177–5182.
13. Wood, I., Roopra, A. & Buckley, N. (1996) *J. Biol. Chem.* **271,** 14221–14225.
14. Lonnerberg, P., Schoenherr, C. J., Anderson, D. J. & Ibanez, C. F. (1996) *J. Biol. Chem.* **271,** 33358–33365.
15. Schoenherr, C. J. & Anderson, D. J. (1995) *Science* **267,** 1360–1363.
16. Thompson, R., Mansour, A., Akil, H. & Watson, S. (1993) *Neuron* **11,** 903–913.
17. Nagalla, S., Gibson, B., Tang, D., Reeve, J. J. & Spindel, E. (1992) *J. Biol. Chem.* **267,** 6916–6922.
18. Bourachot, B., Yaniv, M. & Herbomel, P. (1989) *J. Virol.* **63,** 2567–2577.
19. Lamb, N. J. C., Fernandez, A., Tourkine, N., Jeanteur, P. & Blanchard, J.-M. (1990) *Cell* **61,** 485–496.
20. Purnell, B. A. & Gilmour, D. S. (1993) *Mol. Cell. Biol.* **13,** 2593–2603.
21. Chong, J. H. A., Tapiaramirez, J., Kim, S., Toledoaral, J. J., Zheng, Y. C., Boutros, M. C., Altshuller, Y. M., Frohman, M. A., Kraner, S. D. & Mandel, G. (1995) *Cell* **80,** 949–957.
22. Scholl, T., Stevens, M. B., Mahanta, S. & Strominger, J. L. (1996) *J. Immunol.* **156,** 1448–1457.
23. Atouf, F., Czernichow, P. & Scharfmann, R. (1997) *J. Biol. Chem.* **272,** 1929–1934.
24. Takeda, S., North, D., Diagana, T., Miyagoe, Y., Lakich, M. & Whalen, R. (1995) *J. Biol. Chem.* **270,** 15664–15670.
25. Hollmann, M., O'Shea-Greenfield, A., Rogers, S. & Heinemann, S. (1989) *Nature (London)* **342,** 643–648.
26. Afar, R., Silverman, R., Aguanno, A. & Albert, V. R. (1996) *Mol. Brain Res.* **36,** 79–92.
27. Salton, S., Fischberg, D. & Dong, K. (1991) *Mol. Cell. Biol.* **11,** 2335–2349.
28. Nishimura, E., Sasaki, K., Maruyama, K., Tsukada, T. & Yamaguchi, K. (1996) *Neurosci. Lett.* **211,** 101–104.
29. Shi, Y., Seto, E., Chang, L. & Shenk, T. (1991) *Cell* **67,** 377–388.
30. Sauer, F., Fondell, J., Ohkuma, Y., Roeder, R. & Jackle, H. (1995) *Nature (London)* **375,** 162–164.
31. Roberts, S. & Green, M. (1995) *Nature (London)* **375,** 105–106.
32. Jiang, J., Cai, H., Zhou, Q. & Levine, M. (1993) *EMBO J.* **12,** 3201–3209.