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Biochemistry

A single zinc finger motif in the silencing factor REST represses the neural-specific type II sodium channel promoter

(nervous system/transcription/repressors)

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ABSTRACT **The type II voltage-dependent sodium channel is present in neuronal cells, where it mediates the propagation of nerve impulses. Restricted expression of the type II sodium channel gene to neurons is due, at least in part, to binding of the repressor protein REST (also termed NRSF or XBR) to the RE1 (also called NRSE) sequence in the type II sodium channel gene. Previous studies have shown that a domain in REST containing eight GL1-Krüppel zinc finger motifs mediates DNA binding. Deletional and GAL4-fusion gene analyses now reveal repressor domains that lie outside of the DNA-binding domain in both the amino and carboxyl termini of REST. Mutational analysis further identifies a single zinc finger motif in the carboxyl-terminal domain as being essential for repressing type II sodium channel reporter genes. These studies reveal two domains in REST that may mediate interactions with other proteins involved in restricting expression of a large set of genes to the vertebrate nervous system.**

The ability to generate action potentials is often due to the presence of voltage-dependent sodium channels in the plasma membranes of the excitable cells. Sodium channels are encoded by a large multigene family, and members of this family are structurally distinct and expressed in a tissue-specific manner (reviewed in ref. 1). The type II sodium channel gene (2, 3) is expressed to high levels exclusively in neurons in the central nervous system (4, 5). Because of this selective expression pattern, the type II sodium channel has provided an excellent model for studies of the mechanisms regulating neural-specific gene expression.

Type II sodium channel reporter genes containing 1050 bp of 5′ flanking sequence are expressed in neuronal cell lines but not in nonneuronal cells, consistent with expression of the endogenous gene. Deletional analysis has identified a 23-bp element in the type II sodium channel regulatory region, termed repressor element 1 (RE1), that prevents expression of type II reporter genes in nonneuronal cell types (6, 7). Removal of the RE1 results in approximately 80-fold derepression of the type II sodium channel reporter gene specifically in nonneuronal cell types. Repressor elements with sequences and functional properties similar to those of the type II sodium channel RE1 are present in the regulatory region of several other genes expressed exclusively in the nervous system (reviewed in ref. 8), including SCG10 (9, 10), synapsin (11), the β1 subunit of the nicotinic acetylcholine receptor (12), the muscarinc M4 receptor (13, 14), and neural–glial cell adhesion molecule (15). The widespread occurrence of RE1-like sequences in different genes suggests a more global role of repression in restricting gene expression to the nervous system.

Recombinant RE1-silencing transcription factor (REST) is sufficient to repress reporter genes containing RE1-like target sequences in cotransfection analyses of neuronal cells (8, 16, 17). The deduced primary structure of REST does not reveal any amino acid homologies which would point to repressor domains. However, previous studies suggested that the DNA-binding and repressor domains of REST were physically distinct. The REST DNA-binding domain was identified originally, in a genetic screen in yeast, as a cluster of eight GL1-Krüppel class C_2H_2 (Cys_2His_2) zinc fingers (16). Expression in skeletal muscle cells of a portion of REST containing these zinc fingers resulted in derepression of cotransfected type II reporter genes (16). This result suggested that the amino-terminal zinc fingers were acting as a dominant negative mutant by interfering with binding of the endogenous REST protein to the RE1 target site. The result further suggested that the domains required for repression must be located elsewhere in the molecule.

It is clear, from studies of both prokaryotes and eukaryotes, that several different mechanisms have evolved for repressing gene expression. Despite the importance of negative gene regulation, the molecular components required for this mechanism are only beginning to emerge. REST-mediated repression is cell-type specific, and REST represses minimal promoters that do not require activators for transcription (6, 9). Thus, REST provides an excellent model for studying the molecular basis of this class of repressors. In this study we sought to perform a structure–function analysis of REST for the purpose of elucidating potentially important domains by which REST might interact with the transcriptional machinery.

We have identified, using a deletional and GAL4-fusion gene approach, two distinct repressor domains in REST. As predicted by the earlier studies, expression of the DNA-binding domain alone in neuronal PC12 cells did not result in repression of type II reporter genes. Rather, distinct domains in the amino and carboxyl termini were identified that were each partially required and sufficient to repress the type II promoter. The carboxyl-terminal domain contains a predicted zinc finger motif, and mutations that destroy the finger structure abolish repression. It is likely that the two REST domains

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Abbreviations: RE1, repressor element 1; REST, RE1-silencing transcription factor; NRSF, neural-restrictive silencing factor; UAS, upstream activating sequence; CMV, cytomegalovirus; CAT, chloramphenicol acetyltransferase.

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are sites of interactions with other factors that are required for repression of the set of genes containing RE1 sequences.

METHODS

Plasmid Constructions. The plasmid REEX1 (amino acids 1–1097) is a derivative of the plasmid REST-EXPRESS (16) generated by subcloning a 4.03-kb *Eco*RI fragment containing the entire REST coding sequence into the *Eco*RI site of pcDNAI-Amp (Invitrogen). Constructs encoding partial segments of the REST protein were generated as follows: A partial cDNA clone termed p73 (amino acids 73–545), containing the deduced eight zinc fingers of the DNA-binding domain, has been described (16). REEX21 (amino acids 73–636) was created by a three-way ligation of a *Hin*dIII/*Hin*cII fragment of p73 with a *Hin*cII/*Sph*I fragment of REEX1 between the *Hin*dIII and *Sph*I sites of pcDNAI-Amp. REEX21 extends p73 by 91 amino acids. REEX7, containing an internal deletion between amino acids 636 and 786, was constructed by a three-way ligation of an *Eco*RI/blunted *Sph*I fragment of REEX1 with a blunted *Bst*XI/*Xba*I fragment of REEX1 inserted between the *Eco*RI and *Xba*I sites of pc DNAI-Amp. REEX9 (amino acids 1–1036) was generated by ligation of an *Eco*RI/*Sph*I fragment of REEX1 and a *Sph*I/*Eae*I fragment of REEX1 between the *Eco*RI and *Not*I sites of pcDNAI-Amp. REEX8 (amino acids 73–1097) was created by subcloning a *Pvu*II/*Xba*I fragment of REEX1 between the *Eco*RV and *Xba*I sites of pcDNAI-Amp. The orientation of all recombinant REST constructs was confirmed by restriction analysis, and REST deletional mutants were also checked by sequence analysis to ensure that frameshifts had not occurred.

The plasmid pSG424 containing the GAL4 DNA-binding domain (amino acids 1–147) was obtained from Stan Fields (University of Washington, Seattle). To construct GAL4-N1, the amino-terminal sequences of REST (amino acids 1–83) were obtained by the polymerase chain reaction (PCR) using REEX1 as the template. The PCR products were digested with *Bam*HI and *Kpn*I restriction enzymes and subcloned into the pSG424 vector. GAL4-REEX1 was generated by three-way ligation of a *Bam*HI/*Pvu*II fragment of GAL4-N1 with a *Pvu*II/*Xba*I fragment of REEX1 between the *Bam*HI and *Xba*I sites of pSG424. GAL4-p73 (amino acids 62–545 of REST) was generated by first ligating a *Pvu*II/*Xba*I fragment of GAL4-REEX1 between the *Sma*I and *Xba*I sites of pSG424, generating GAL4-REEX8. Second, a *Cla*I/*Xba*I fragment of p73 was ligated between the *Cla*I/*Xba*I sites of GAL4-REEX8, resulting in GAL4-p73. To construct GAL4-C3, REST amino acids from 1008 to 1097, containing the single zinc finger, were amplified by the PCR and the fragment was subcloned into the pSG424 backbone at the *Bam*HI site. All of the GAL4-REST fusions were sequenced across the GAL4-REST junction to ensure that the inserts were in frame with GAL4. All constructs generated by the PCR were fully sequenced to ensure that mutations/deletions had occurred during the amplification reaction.

The RE1-containing type II sodium channel-chloramphenicol acetyltransferase (CAT) reporter gene, pSDK7, has been described previously (7). The upstream activating sequence (UAS) type II CAT reporter gene was generated by substituting the 23-nucleotide type II RE1 sequence in the CAT reporter plasmid for five copies of the UAS from the plasmid pGAL4-TKCAT provided by Thomas Shenk, Princeton University (18).

Site-Directed Mutagenesis. To generate a mutant REST molecule with an amino acid change in the predicted carboxyl-terminal zinc finger motif, a commercial DNA mutagenesis kit was used (Morph, 5 Prime → 3 Prime). An oligonucleotide encoding a mutation in a cysteine residue of the zinc finger was synthesized. This oligonucleotide, 5′-GCGGCTAAGG-GAGATTTTGTT**C**GTATCTTCTGTGATCG-3′, was used to construct the mutant GAL4-C3M1. The fragment was checked by sequence analysis. (The boldface letter represents the change from wild-type sequence.) The mutation results in the substitution of an arginine residue for a cysteine. To generate an intact REST molecule containing the mutation in the carboxyl-terminal zinc finger, an *Eco*RI/*Eag*I fragment of REEX1 and an *Eae*I/*Xba*I fragment of GAL4-C3M1 were ligated into the pcDNA1-Amp vector at the *Eco*RI and *Xba*I sites.

Transient Transfections and CAT Assays. PC12 cells were grown as described previously (19). COS-1 cells were grown in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% bovine calf serum (HyClone). COS-1 cells were transfected with 8 μg of plasmid DNA by treatment with calcium phosphate (20). For the transfections shown in Fig. 1, PC12 cells were electroporated with mixtures of 10 μg of reporter plasmid containing either 5 μg of empty pcDNAI-Amp vector or 10 μg of pcDNAI-Amp plasmids containing REST cDNA inserts. For the transfections shown in Fig. 2, PC12 cells were electroporated with mixtures of 10 μg of reporter plasmid and 5 μg of pSG424 or equimolar (to 5 μg of pSG424) amounts of GAL4-REST fusion cDNA constructs. To all mixtures, pBluescript II SK (Stratagene) was added to bring the final amount of DNA to 20 μg. For the transfections shown in Fig. 3, 10 μg of reporter plasmid was mixed with either 1 μg of pcDNAI-Amp or an equimolar amount of pcDNAI-Amp containing REST inserts. The mixture of cells and DNA was chilled on ice for 5–10 min before electroporation (250 mV, 960 mF). Following electroporation the cells were chilled on ice for a further 10 min before plating in 100-mm dishes. Medium was changed 24 hr after transfection and cells were harvested after 48 hr. The protein concentration of the cell lysates was determined with the micro BCA protein assay reagent (Pierce) in microtiter plates. Assays for CAT activity in lysates of the harvested cells were performed as described previously (7). Relative activity of the extracts was calculated by determining the percentage of acetylated chloramphenicol, using a Molecular Dynamics PhosphorImager. For each construct at least two different preparations of plasmid DNA were used (Qiagen from Qiagen or JetStar from Genomed). In each experiment, constructs were transfected in duplicate and different experiments were performed several times as noted in *Results*.

Western Blot Analyses. Nuclear extract preparation and Western blotting of COS-1 cells transfected with the appropriate cDNA constructs were performed as described previously (16), and the samples were solubilized in Laemmli sample buffer. After separation on reducing SDS/polyacrylamide gels, either 7% or 12% polyacrylamide, proteins were transferred onto nitrocellulose membranes. Blots were incubated either with a polyclonal anti-GAL4 antibody (Upstate Biotechnology) or an affinity-purified polyclonal anti-REST antibody. The antibodies were visualized by using the ECL detection method (NEN).

RESULTS

Two Distinct Domains in REST Are Involved in Repression of Type II Sodium Channel Reporter Genes in PC12 Cells. Although no known repressor consensus sequences were apparent in the deduced primary sequence of REST, three distinct domains, depicted in Fig. 1, were identified. These domains, from amino terminus to carboxyl terminus, consisted of the following: (*i*) a cluster of eight GLI-Krüppel type zinc fingers constituting the DNA-binding domain (16, 17), (*ii*) a novel reiterated proline-rich motif, and (*iii*) a single C_2H_2 zinc finger motif in the carboxyl terminus. Here, we have generated a family of deletion molecules to examine the potential involvement of these motifs in transcriptional repression by REST. The REST cDNAs were placed under control of the

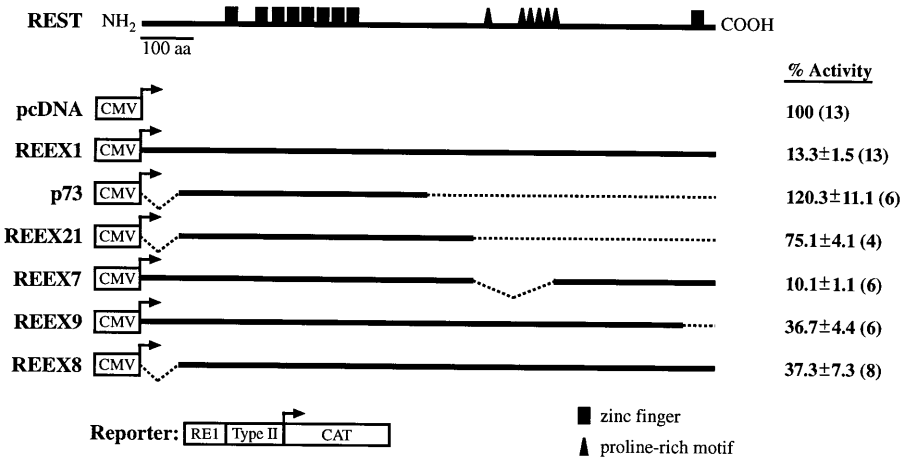

FIG. 1. Domains in the amino and carboxyl termini of REST are required, in part, to mediate repression of type II sodium channel reporter genes. The family of REST molecules expressed under control of the cytomegalovirus (CMV) promoter, in PC12 cells, is shown with respect to the presence of known motifs in the deduced primary structure of REST (top line). The dotted lines indicate the regions in REST that were deleted. A cartoon of the cotransfected reporter gene shows the positions of the type II repressor element 1 (RE1), type II sodium channel promoter (type II) and CAT gene (bottom line). Arrows indicate the start sites for transcription in the expression plasmids. On the right, the percent activity of CAT resulting from transfection of the different constructs is normalized to that from the vector alone, which is set to 100%. Standard errors of the mean and (in parentheses) the numbers of experiments are indicated.

CMV 1E promoter in the mammalian expression vector pcDNA1 (Fig. 1). The constructs were cotransfected into PC12 cells, which do not express significant levels of the endogenous REST gene, along with CAT reporter genes containing the type II sodium channel RE1 sequence upstream of the type II promoter. CAT activity resulting from cotransfection of the type II reporter gene and the empty vector was set to 100%.

Cotransfection of PC12 cells with the type II sodium channel reporter gene and the full-length REST cDNA (REEX1) caused a greater than 7-fold repression of reporter gene expression (13.3% CAT activity; Fig. 1). Because the domain containing the cluster of eight zinc fingers is required for DNA binding to the RE1 sequence (16) this domain must be included in all of the REST deletional mutants. As expected, expression of the REST DNA-binding domain alone (p73) did not result in repression of the type II reporter gene. In fact, CAT activity of this mutant was slightly greater than that of the control value, perhaps representing a slight dominant interfering effect from competition for RE1 binding by the low levels of REST protein that are present in PC12 cells (ref. 16; see also results with GAL4-p73 in Fig. 2*B*). Inclusion of a region adjacent to the DNA-binding domain caused only a modest increase in repressor activity (75% CAT activity; Fig. 1). The domain containing the six reiterated proline-rich motifs was also deleted from the intact REST molecule (REEX7). The removal of this domain did not interfere with repressor activity (Fig. 1), indicating that these motifs are not required for the repressor mechanism.

Expression of a truncated REST molecule lacking the 60 terminal amino acids of REST that includes the lone zinc finger motif (REEX9) resulted in a partial derepression of the type II promoter, causing an approximately 3-fold increase in CAT activity compared with that mediated by the wild-type REEX1 molecule. Thus, of all of the obvious domains revealed by elucidation of the REST primary sequence, only the carboxyl-terminal zinc finger domain exhibited significant repressor activity. However, the requirement for this domain was only partial, suggesting that other domains in REST were also required to mediate repression. In support of this interpretation, a partial cDNA extending from the predicted initiator methionine in NRSF to amino acid 585 also exhibited repressor activity in transient transfection analyses (17). By process of elimination in comparing the predicted structures of the partial NRSF protein and full-length REST/NRSF, another candidate for a repressor domain was the amino terminus of REST that was missing in the DNA-binding domain construct, p73. Results of transfections with a REST mutant that lacks these amino acids (REEX8) showed that the amino-terminal domain was partially required for repression of type II sodium channel reporter gene expression (37.3% CAT activity; Fig. 1). The amount of repression exhibited by the REST mutants deleted in the amino- and carboxyl-terminal domains were roughly equivalent (approximately 3-fold repression of reporter gene activity for each construct).

To determine whether the amino- and carboxyl-terminal domains were sufficient to mediate repression, repressor activity of the individual domains was tested by fusing them in-frame to cDNA coding for the DNA-binding domain of the yeast activator protein GAL4. Correspondingly, in the type II sodium channel reporter gene, the type II RE1 was replaced with five copies of the yeast UAS, the target site for binding by the GAL4 protein. The chimeric cDNAs and UAS reporter genes were then cotransfected into PC12 cells. The activity of the UAS type II reporter gene coexpressed with the GAL4 DNA-binding domain alone was set at 100%.

To validate this system for analyzing REST repressor activity, a chimeric protein containing the full-length REST protein (GAL4-REEX1; Fig. 2*A*) was first introduced into PC12 cells along with the UAS reporter gene. As expected, CAT activity of GAL4-REEX1 was repressed approximately 4-fold compared with control CAT activity (Fig. 2*B*). To determine whether the 83 amino-terminal (GAL4N1; Fig. 2A) or 88 carboxyl-terminal (GAL4C3; Fig. 2*A*) amino acids were sufficient to mediate repression in this system, these domains were also fused in-frame with the GAL4 DNA-binding domain. Expression of these REST domains resulted in dramatic repressor activity, 9.5-fold and 11-fold, respectively (Fig. 2*B*), indicating that they were indeed sufficient to mediate repression of UAS reporter gene expression. In contrast to these results, transfections with the GAL4-p73 fusion gene (Fig. 2*A*), encoding the eight Krüppel type zinc fingers in the DNA-binding domain, did not result in repression of reporter gene activity (Fig. 2*B*).

Mutation of the Single Zinc Finger in the Carboxyl-Terminal Domain Abrogates Repression. Unlike the amino-terminal domain, the carboxyl-terminal domain that was sufficient to repress the type II sodium channel promoter contained a recognizable motif, a single C_2H_2 zinc finger. To test

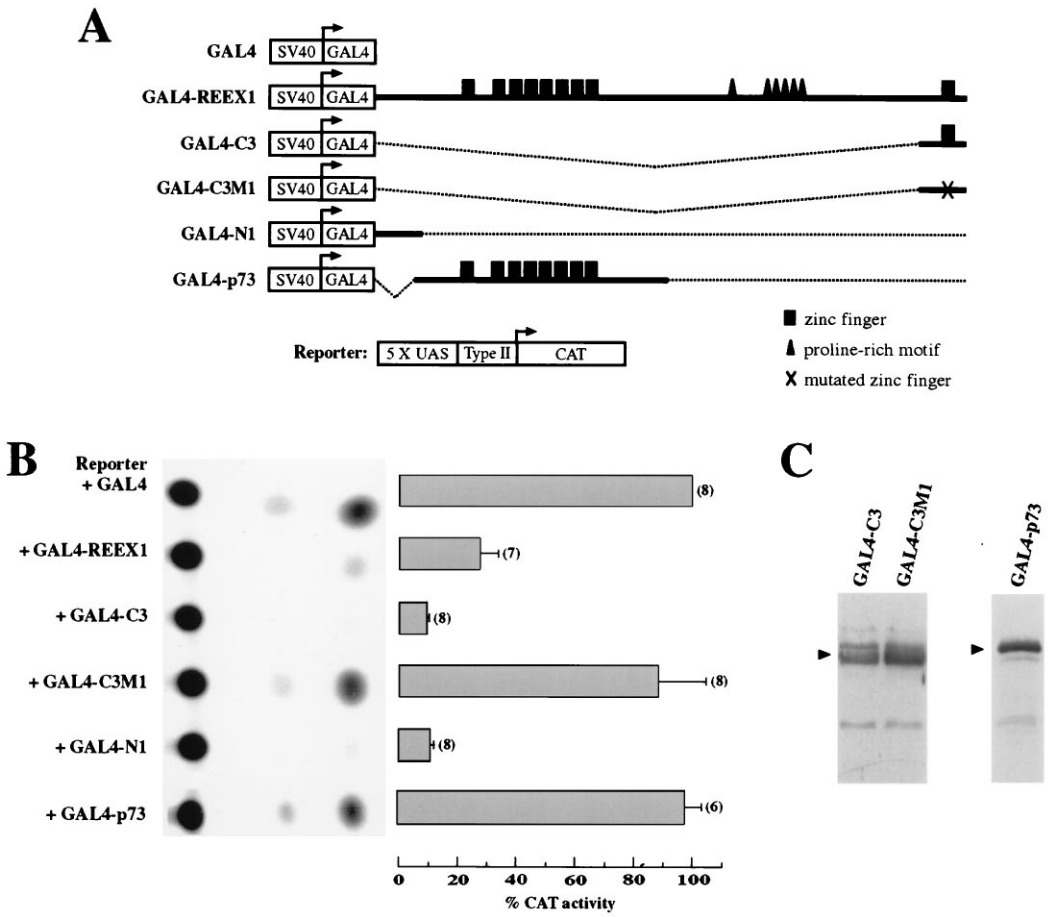

FIG. 2. Domains in the amino and carboxyl termini of REST are sufficient to mediate repression, and a point mutation in the zinc finger motif abrogates repressor activity. (*A*) Schematic representation of the family of GAL4-REST chimerical cDNAs expressed with a UAS type II-CAT reporter gene in transient transfections of PC12 cells. The reporter gene contains five copies of the UAS. The dotted lines indicate regions in REST that were deleted. Arrows indicate start sites for transcription. The carboxyl-terminal (C3), amino-terminal (N1), and mutated carboxyl-terminal (C3M1) fragments of REST are in-frame with the DNA-binding domain of the GAL4 protein. (*B*) (*Left*) Representative autoradiogram showing thin-layer chromatography (TLC) fractionation of acetylated forms of chloramphenicol. Each sample is from a dish of cells transfected transiently with the GAL4-REST chimerical cDNA and the UAS type II reporter gene. Note that the CAT assay with the GAL4-p73 construct was from a different TLC plate. (*Right*) Histogram showing compiled data from independent experiments. Standard errors of the mean and the numbers of experiments are indicated. (*C*) Western blot analysis of COS-1 cells transfected with the indicated GAL4-REST carboxyl-terminal (C3 and C3M1) and GAL4-p73 constructs. The amounts of the expressed proteins (arrowheads) show that the inability of the mutated carboxyl-terminal fragment C3M1 and p73 to repress is not due to instability of the expressed protein.

whether this motif was required for repression by REST, one of the cysteine residues critical for the zinc finger structure was changed to an arginine, and the mutated domain was fused in-frame with the GAL4-DNA-binding domain. The mutated construct (GAL4C3M1) was cotransfected into PC12 cells along with the UAS type II reporter gene (Fig. 2*B*). The zinc finger mutation abolished the repressor activity normally observed with the corresponding wild-type REST domain (the CAT activity was equivalent to that seen by expression of the control GAL4 and GAL4-p73 constructs). Western blot analysis of COS-1 cells transfected with the GAL4 fusion genes indicated that the lack of repression by the mutated carboxyl-terminal zinc finger domain (GAL4-C3M1) and by the REST p73 domain was not due simply to differences in the levels of expressed chimeric proteins (Fig. 2*C*). Repression of reporter gene activity mediated by the GAL4-REST fusion proteins required the REST domains to be tethered to the DNA through the GAL4 DNA-binding domain because transfections of the GAL4-REST constructs with a type II reporter gene lacking an UAS did not result in repression of CAT activity (data not shown).

The above studies indicated that the single zinc finger motif was required for repression mediated by the carboxyl-terminal REST domain. To determine whether the zinc finger motif was also required for repression within the context of the intact REST molecule, the cysteine-to-arginine point mutation was introduced into the full-length REST molecule REEX1 and cotransfected with the RE1-type II sodium channel reporter gene into PC12 cells (Fig. 3). The point mutation in REEX1 (REEX1M1) resulted in a partial derepression of the type II promoter (4.5-fold increase in CAT activity compared with wild-type REEX1; Fig. 3*B*). The residual repressor activity is likely due to the presence of the amino-terminal fragment shown above to be sufficient to partially mediate repression. Transfection of the wild-type and mutant cDNAs into COS-1 cells resulted in the expression of a 200-kDa protein that was detected by an anti-REST antibody (Fig. 3*C*). The reduced repressor activity of mutated REST was not due to reduced accumulation of the protein, because Western blot analysis indicated similar levels of expression of the wild-type and mutant proteins (Fig. 3*C*).

DISCUSSION

The molecular mechanisms responsible for regulating expression of genes in the nervous system are understood poorly.

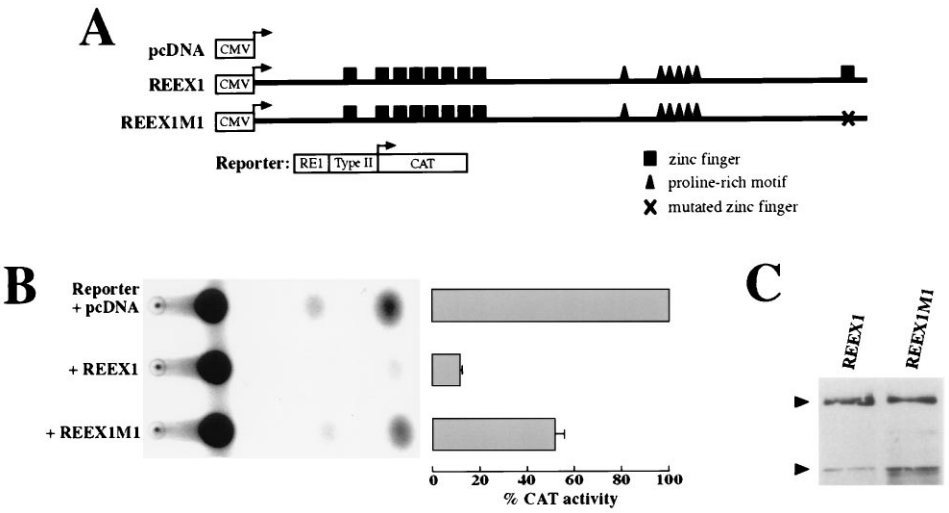

FIG. 3. The single C_2H_2 zinc finger in the carboxyl terminus of REST is sufficient to mediate repression of type II sodium channel reporter genes. (*A*) Schematic representation of the REST expression vector and type II-CAT reporter genes. Relative locations of distinct domains in REST are indicated. (*B*) Representative autoradiogram (*Left*) shows TLC fractionation of acetylated forms of chloramphenicol from PC12 cells cotransfected with the different REST constructs shown and the RE1-type II-CAT reporter gene. Other symbols are the same as in Fig. 1. A histogram (*Right*) shows a compilation of CAT activity from four independent experiments using the REST constructs indicated for the autoradiogram. Standard errors of the mean are shown. (*C*) Western blot analysis of COS cells transfected with the wild-type (REEX1) and mutated (REEX1M1) cDNAs. Upper arrowhead denotes overexpressed REST protein (200 kDa) and lower arrowhead denotes cross-reacting endogenous protein migrating at 116 kDa (16).

However, recent studies have shown that at least one of these mechanisms involves transcriptional repression mediated by the DNA-binding protein REST. The discovery that REST is also involved in regulating the expression of many other genes expressed in the nervous system, through a common RE1-like genetic element, underscores the importance of elucidating the molecular mechanism by which REST repression is mediated.

Eukaryotic transcription can be prevented by mechanisms that are dependent upon (silencing) or independent of (repression) chromatin structure (for review see ref. 21). The observation that REST can inhibit reporter gene expression in transient transfection analyses with plasmid DNA suggests that chromatin remodeling is not required for its ability to block transcription, and classifies REST as a repressor. Two well characterized domains have been shown to mediate the activities of other repressor proteins, a domain that is rich in alanine residues (22–24), and a Krüppel-associated box A (KRAB-A) domain, rich in charged amino acids, that is present in a large number of zinc finger proteins (25, 26). The deduced primary structure of full-length REST (refs. 16 and 27, and D. Anderson, personal communication) does not contain either of these motifs.

The cluster of eight GL1- Krüppel type zinc fingers in REST binds to the type II RE1 sequence *in vivo* and *in vitro* (16, 17). Despite the ability to bind DNA, they are not sufficient to mediate repression. An additional C_2H_2 zinc finger motif resides in the deduced carboxyl terminus of REST. The deletional and GAL4 fusion gene analyses performed in this study indicated that, unlike the DNA-binding domain, this domain was partially required and sufficient to mediate repression of type II sodium channel reporter genes. In fact, the amount of repression mediated by the GAL4-carboxyl-terminal fusion protein was even greater than that mediated by repression of the GAL4-REST chimera containing the entire REST sequence. It is possible that the zinc finger motif in the isolated carboxyl-terminal domain is more accessible to other components involved in the repression mechanism than when embedded in the intact REST molecule. To exclude the possibility that all small fragments fused to GAL4 will mediate repression in this system, we examined two additional REST fragments of a size similar to that of the carboxyl-terminal domain. The small GAL4-REST fusion proteins do not exhibit repressor activity (data not shown). Furthermore, the carboxyl-terminal domain containing a single point mutation in the zinc finger motif also does not exhibit repressor activity.

Zinc finger motifs in proteins are usually associated with DNA binding (for review see ref. 28). The zinc fingers can also mediate protein–protein interactions (29–34), and some of the proteins that contain zinc finger motifs are transcriptional repressors. However, to our knowledge, REST is the first example whereby a zinc finger structure is required to mediate repression. For example, in the transcription factor YY1, although an identified repressor domain contained two zinc fingers, mutational analysis indicated that the structures of these zinc fingers were not required for repression (35). It may be that the zinc finger motif in REST is sufficient to mediate repression, although flanking amino acid sequences may also contribute to the ability of the minimal 23 amino acid motif to repress. Interestingly, in the transcription factor TFIIIA, the linker sequence characteristic of Krüppel type zinc fingers has been shown to confer high-affinity DNA binding on the zinc finger domain (36). This linker sequence is present in the Krüppel type zinc fingers that constitute the DNA-binding domain in REST. The zinc finger motif in the carboxyl-terminal domain of REST that mediates repression, as a single structure, does not have this linker sequence and does not appear to bind DNA. For example, in transient transfection analysis, chimeric GAL4 proteins containing the carboxyl-terminal domain of REST, and thus the zinc finger, do not repress type II sodium channel reporter genes containing the RE1 sequence in place of the UAS (J.T.-R. and G.M., unpublished results). Although we cannot formally exclude the possibility that this zinc finger motif binds to DNA (but see ref. 27), it does not appear to bind to the type II RE1 sequence.

The deletional and fusion gene studies herein have revealed the presence of two distinct repressor domains located at opposite ends of the REST molecule. Indeed, the REST amino-terminal domain fused to GAL4 is as effective in mediating repression of the type II promoter as is the carboxyl-terminal domain. Further, like the zinc finger domain, the amino-terminal domain is also partially required for repres-

sion. No obvious motifs are present within the amino-terminal sequences. However, future studies, such as comparisons with REST homologues in other species, may help clarify the functional motifs.

Many repressor complexes consist of a DNA-binding protein interacting with corepressors. Examples of such complexes are thyroid hormone and retinoic acid receptors and the TRACs (37, 38), Mad/Max/sin3 (39), yeast TUP1/SSN6 and several different DNA-binding proteins (e.g., see ref. 40), the immediate early proteins NAB1/NGF1A/KROX20 (41), and *Drosophila* Hairy-related proteins and Groucho (42, 43). It is likely that the amino-terminal and zinc finger domains now identified in REST interact with corepressor proteins or with proteins that are part of the initiation complex.

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