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Neural-specific expression, genomic structure, and chromosomal localization of the gene encoding the zinc-finger transcription factor NGFI-C

SETH D. CROSBY*, ROSALIE ANN VEILE†, HELEN DONIS-KELLER†, JAY M. BARABAN‡, RATAN V. BHAT‡, KELLI S. SIMBURGER*, AND JEFFREY MILBRANDT*§

*Departments of Pathology and Internal Medicine, Division of Laboratory Medicine, and †Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110; and ‡Department of Neuroscience, WBSB 908, Johns Hopkins University School of Medicine, Baltimore, MD 21205

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ABSTRACT **The nerve growth factor-induced clone C (NGFI-C) gene encodes a zinc-finger transcription factor that is rapidly induced by nerve growth factor in rat pheochromocytoma PC12 cells and by seizure in brain. NGFI-C is closely related to the previously described early response genes, nerve growth factor-induced clone A (NGFI-A or *EGR1*), *EGR2*, and *EGR3*. These four early response (immediate early) proteins all contain very similar zinc-finger DNA binding domains; in addition, analysis of the non-zinc-finger region revealed that they share an additional five highly homologous subdomains, four of which are within the amino terminus. The 5′ flanking region of NGFI-C contains several cAMP response elements but does not contain any serum-response elements or CArG boxes [CC(A/T)$_6$GG], cis-acting elements commonly involved in early response gene regulation. NGFI-C mRNA was detected in neural tissues of postnatal animals, but no expression was found in rat embryos. *In situ* hybridization demonstrated that NGFI-C is rapidly induced in the dentate gyrus of the hippocampus after seizure, but in contrast to NGFI-A, increases in NGFI-C mRNA were not detected in the overlying cortex. By using fluorescence *in situ* hybridization, NGFI-C was localized to human chromosome 2p13. This region contains a constitutive fragile site that is associated with chromosomal breakpoints and translocations characteristic of some chronic lymphocytic leukemias.**

When eukaryotic cells are stimulated to undergo mitogenesis or differentiation, the expression of a small subset of genes, termed early response or immediate early genes (1), is rapidly activated. Many early response genes encode transcriptional regulators (2, 3), for example nerve growth factor-induced clones C and A [NGFI-C and NGFI-A (also called *EGR1* and *zif268*, respectively)], *Krox-20/EGR2*, and *EGR3*, encode transcription factors whose DNA-binding domains are highly related. These domains consist of three zinc fingers and recognize the DNA element GCGGGGGCG (4) from which each of these proteins is capable of activating transcription (4–6).

The NGFI-C cDNA was originally isolated from nerve growth factor (NGF)-stimulated rat pheochromocytoma PC12 cells by using degenerate probes corresponding to the zinc-finger region (7). RNA blot analysis showed that NGFI-C was rapidly activated in PC12 cells treated with NGF and by seizure in rat brain. We now report the isolation and nucleotide sequence of the rat NGFI-C gene,¶ which is structurally similar to the NGFI-A, *EGR2* (the human homolog of *Krox-20*), and *EGR3* loci. Analysis of the amino acid sequences of these four proteins revealed that they share five regions of homology in addition to their DNA-binding domains. At birth no NGFI-C expression was detected in the rat, but NGFI-C mRNA levels increase gradually during the first 3 weeks of life, plateauing at a level similar to that observed in the adult. In the adult rat, NGFI-C expression is restricted to the brain and other neural tissues. Like other early response genes, NGFI-C expression was detected primarily in the dentate gyrus after a seizure induced by electroconvulsive treatment (ECT). Chromosomal *in situ* hybridization was used to demonstrate that NGFI-C is located at human chromosome 2p13, a region involved in a variant of chronic lymphocytic leukemia.

METHODS

Genomic Library Screening. Genomic clones containing either the rat (rλ4A) or human (hλ3) NGFI-C genes were obtained by screening the respective genomic libraries with ^{32}P-labeled rat NGFI-C cDNA or an 800-nucleotide (nt) fragment of the human NGFI-C gene (see below) (8). The rat genomic EMBL3 library was a gift from R. Hynes (Massachusetts Institute of Technology) and the human genomic λ-FIX library was obtained from Stratagene.

Primer-Extension and S1 Nuclease Protection Reactions. RNA was isolated from cells or tissues (9). Both S1 and primer-extension reactions were performed with 50 μg of total RNA as described (10). The products of both reactions were analyzed on 8% polyacrylamide/urea sequencing gels. A sequencing reaction mixture was used as a marker to determine fragment lengths.

Gene Mapping by *in Situ* Hybridization. A fragment of the human NGFI-C gene was isolated using the PCR with primers derived from the rat sequence (nt 275–294 and nt 1051–1070). Two human-NGFI-C-specific primers (5′-GGAGCTATAGAATAGGGGC and 5′-CATACAGGCACTGCGAGGGG) were synthesized and used in a PCR assay to screen two panels of DNA derived from human–rodent somatic cell hybrids (Bios, La Jolla, CA, and NIGMS Mutant Cell Repository, Camden, NJ) (11).

Fluorescence *in situ* hybridization was performed essentially as described (12). Human prometaphase chromosome spreads were prepared from cultured phytohemagglutinin-stimulated peripheral blood lymphocytes from a male with a normal karyotype. Extended chromosomes were produced by colchicine treatment (37). The (hλ3) NGFI-C clone, which contains a 12-kilobase human genomic DNA insert, was

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Abbreviations: NGF, nerve growth factor; NGFI-A, NGFI-C, etc., NGF-induced clones A, C, etc.; ECT, electroconvulsive treatment; nt, nucleotide(s); E, embryonic day; P, postnatal day.

§To whom reprint requests should be addressed at: Departments of Pathology and Internal Medicine, Washington University School of Medicine, Box 8118, 660 South Euclid Avenue, St. Louis, MO 63110.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M92433).

labeled with biotin-11-dUTP by nick translation (13), and each chromosomal spread was hybridized with 150 ng of the biotin-labeled NGFI-C probe (13). For fluorochrome detection, slides were incubated with fluorescein-conjugated avidin DCS (Vector Laboratories) at 5 μg/ml, amplified by incubation with fluorescein-conjugated goat anti-avidin D antibodies (Vector Laboratories) at 5 μg/ml, and counterstained with 4,6-diamidino-2-phenylindole dihydrochloride at 200 ng/ml and propidium iodide at 200 ng/ml, which were present in the final wash solution. Cytogenetic banding patterns were visualized by staining with Giemsa after fluorescent hybridization.

***In Situ* Hybridization on Tissues.** Seizures that resulted in a generalized tonic–clonic convulsion with hindlimb extension were induced in male Sprague–Dawley rats by electric shock as described (14). Rats were sacrificed 0, 30, 60, 120, 180, and 480 min after seizure, and the brains of control and treated rats were processed as described (14). The NGFI-C antisense probe (nt 330–1 of the NGFI-C cDNA) was transcribed using uridine 5′-[α-[^{35}S]thio]triphosphate (Amersham) and RNA probe transcription reagents (Promega) as described by the manufacturer's protocol. NGFI-A probes were synthesized from the 3′ end of the cDNA after linearization with *Pst* I. *In situ* hybridization with NGFI-C or NGFI-A was carried out as described (15), except that washes were performed in 0.2× or 2× standard saline citrate (SSC) containing RNase (10 μg/ml) at 32°C followed by a 30-min wash in 0.2× or 2× SSC at room temperature. Similar results were obtained with either salt condition. In control experiments, the appropriate sense probes were hybridized to similar tissue sections to ensure the specificity of the detected signal.

RESULTS

Isolation and Characterization of the Rat NGFI-C Gene. A rat genomic library was screened using ^{32}P-labeled NGFI-C cDNA. Southern blot analysis demonstrated that one positive clone, rλ4A, contained the entire NGFI-C gene. The nucleotide sequence of the entire NGFI-C gene and 1602 nt of the 5′ flanking region was determined (Fig. 1). A comparison of the NGFI-C genomic and cDNA sequences revealed that the NGFI-C gene is 2452 nt long and is interrupted by a single 316-nt intron located at nt 325 (7). The presumed translation start site is located at nt 192 of the first exon and all three zinc fingers are encoded within the second exon.

To determine the transcriptional start site of the NGFI-C gene, primer extension was carried out with a ^{32}P-labeled antisense oligonucleotide primer extending from nt 49 to nt 20 of the NGFI-C cDNA (7). This oligonucleotide was hybridized to RNA samples in which NGFI-C mRNA was known to be present (PC12 cells treated with NGF and cycloheximide) or absent (uninduced PC12 cells and spleen) and extension reactions were performed using reverse transcriptase. A single 100-nt extension product was detected only in the sample obtained from NGF/cycloheximide-treated PC12 cells (Fig. 2). An S1 nuclease protection experiment was

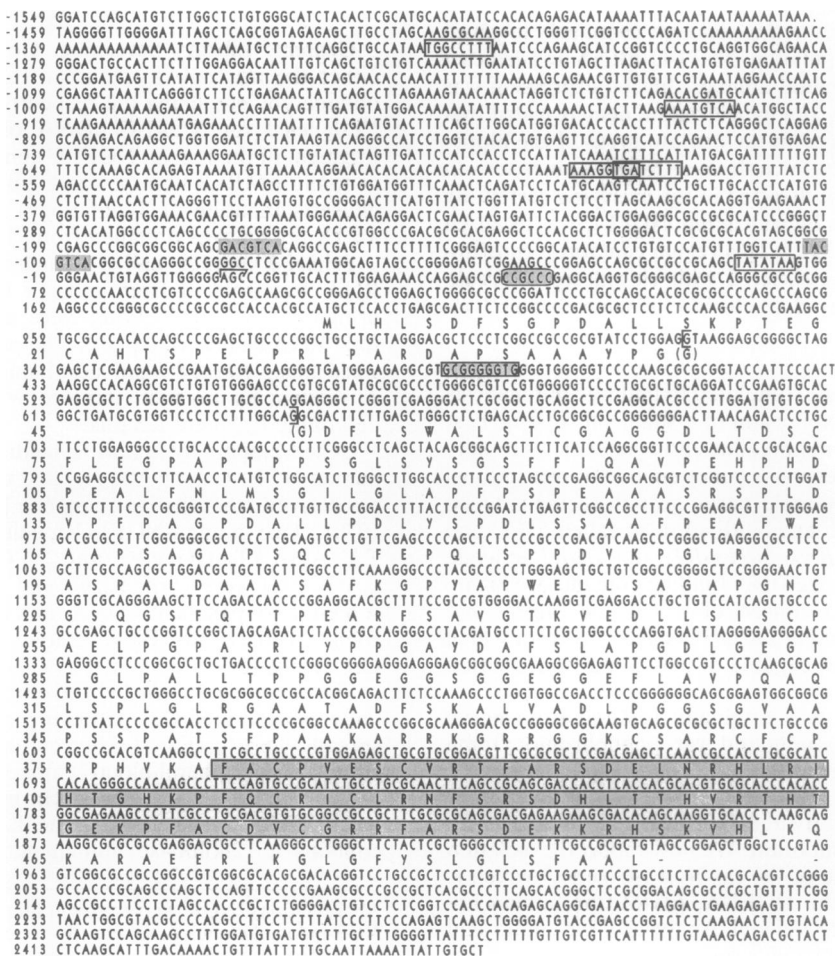

FIG. 1. Nucleotide sequence of the rat NGFI-C gene and 5′ flanking region. The site of transcriptional initiation is at position 1. The intron is bracketed and the GCGGGGGTG element is boxed and shaded. The residues constituting the zinc-finger region are boxed and shaded. Sequence elements within the 5′ flanking region are denoted as follows: cAMP responsive elements (shaded), NGFI-B response elements (boxed), TATA box (boxed), and an Sp1 site (boxed and shaded). The numbers on the left refer to the nucleotide sequence using the transcription start site as nt +1. The numbered amino acid sequence of the NGFI-C protein is shown beneath the nucleotide sequence.

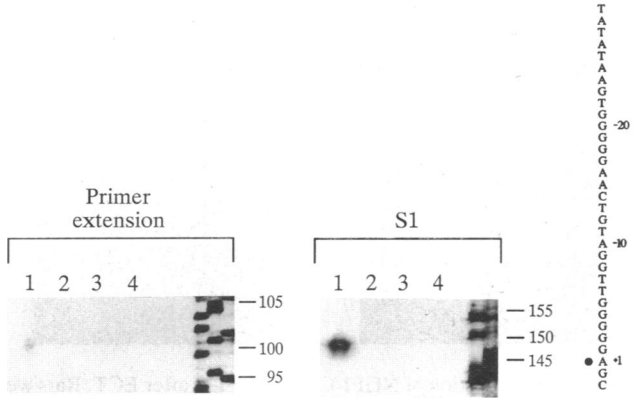

FIG. 2. Analysis of the rat NGFI-C transcriptional start site. S1 nuclease protection and primer-extension assays were performed on total RNA isolated from PC12 cells treated with NGF in the presence of cycloheximide for 3 hr (lanes 1), uninduced PC12 cells (lanes 2), spleen (lanes 3), and yeast tRNA (lanes 4). A known sequence was used for size markers. The nucleotide sequence near the transcription initiation site (indicated by a dot), which includes the TATA box, is shown.

performed to confirm the primer-extension result. A 362-nt *Bss*HII genomic fragment that extended to nt 95 of the NGFI-C cDNA was end-labeled and hybridized with RNA samples from the same sources as those used in the primer extension. Digestion with S1 nuclease resulted in a 146-nt protected fragment that was again only detected in RNA from NGF/cycloheximide-treated PC12 cells (Fig. 2). These results indicated that transcription of NGFI-C mRNA begins at a single site 51 nt upstream of the 5′ end of the original cDNA and 24 nt downstream from the TATA box identified in the NGFI-C 5′ flanking region.

The sequence of the NGFI-C promoter region is shown in Fig. 1. Sequence analysis of the promoter region revealed the presence of two cAMP-responsive elements at nt −112 and −180 and four NGFI-B-like response elements (16) at nt −1326, −938, −589, and −584. A "TATA" box is present at nt −24 and a "GC" box is present within the first exon at nt +34. Interestingly, we also located an element, GCGGGGGTG, within the intron of the NGFI-C gene at nt 387 that is a binding site for NGFI-C and other members of its gene family (A. Swirnoff and J.M., unpublished observations). Sequences resembling a serum-response element or a CArG box [CC(A/T)$_6$ GG], which are often found in the 5′ flanking regions of early response genes, were not found in the NGFI-C promoter.

Relationship of NGFI-C to Other Members of This Gene Family. The identification and sequencing of four members of this family allowed us to search outside the zinc-finger DNA binding domains, whose homology has been described, for additional regions of homology. By using amino acid sequence alignment programs (PALIGN and CLUSTAL, PC/GENE; IntelliGenetics), we found that NGFI-C, NGFI-A, *EGR2*, and *EGR3* share five regions of homology outside of the DNA-binding domain. Four of these are located on the amino-terminal side of the zinc fingers, a region known to contain the transcriptional activation domain of NGFI-A (M. Russo and J.M., unpublished observations). Three additional regions of homology shared only by NGFI-A, *EGR2*, and *EGR3* were also found (Fig. 3).

Chromosomal Location of the Human NGFI-C Locus. To localize the human homologue of NGFI-C, we first isolated a genomic fragment of human NGFI-C (see *Methods*). By using the PCR, primers corresponding to rat NGFI-C were utilized under low-stringency conditions with total human placental DNA to synthesize an 0.8-kilobase fragment.

Sequence analysis of this fragment (hc800) showed it contained an open reading frame that predicted an amino acid

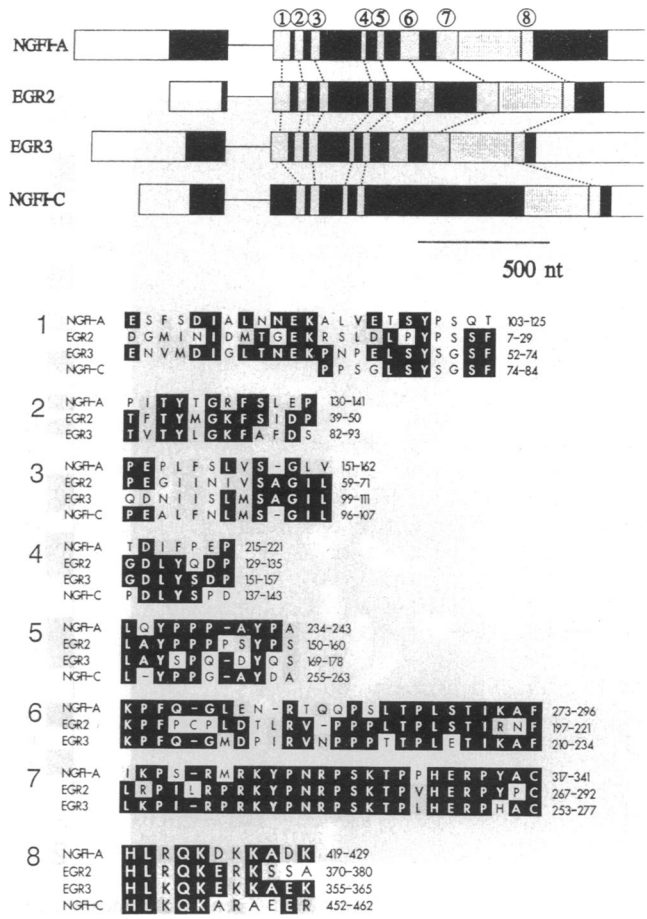

FIG. 3. Sequence alignment of the rat NGFI-C with other members of its gene family. The NGFI-C, NGFI-A, *EGR2*, and *EGR3* genes are displayed at the top. The exons (drawn to scale) are represented by boxes (open, noncoding regions; solid, coding regions; vertical hatch, zinc-finger DNA binding domain); introns are shown as solid lines (not to scale). The regions of homology are depicted as shaded boxes numbered 1 to 8 and the corresponding regions in each protein are joined by dashed lines. The aligned amino acid sequences from each protein within the eight numbered regions of homology are shown below. Identical residues are denoted by white letters on black squares; similar residues are denoted by black letters on shaded squares; and nonconserved residues are denoted by black letters on a white background. The relative positions of the residues are indicated to the right of each row.

sequence that was 88% identical to residues 147–176 of the rat amino acid sequence. Oligonucleotides were designed and used for screening of human–mouse somatic cell hybrids by the PCR. Two such panels were screened, and concordance was observed for the presence of NGFI-C and human chromosome 2 in each panel (data not shown). This result was confirmed by Southern blot analysis using ^{32}P-labeled hc800 as the probe on DNA isolated from the somatic hybrid cell line GM10826B, a CHO cell line that contains only human chromosome 2 (data not shown).

Fluorescence *in situ* hybridization was employed to subregionally localize the NGFI-C gene on chromosome 2 essentially as described by Lichter *et al.* (12). A genomic clone, hλ3, containing the human NGFI-C gene was labeled with biotin and hybridized to metaphase spreads of human chromosomes. The labeled DNA was detected with fluorescein isothiocyanate-conjugated avidin DCS and amplified using goat anti-avidin D antibodies. Fig. 4 shows specific hybridization of the biotin-labeled NGFI-C probe to chromosome 2 (two metaphase chromosomes 2 are evident). No consistent secondary hybridization was observed in more than 30 meta-

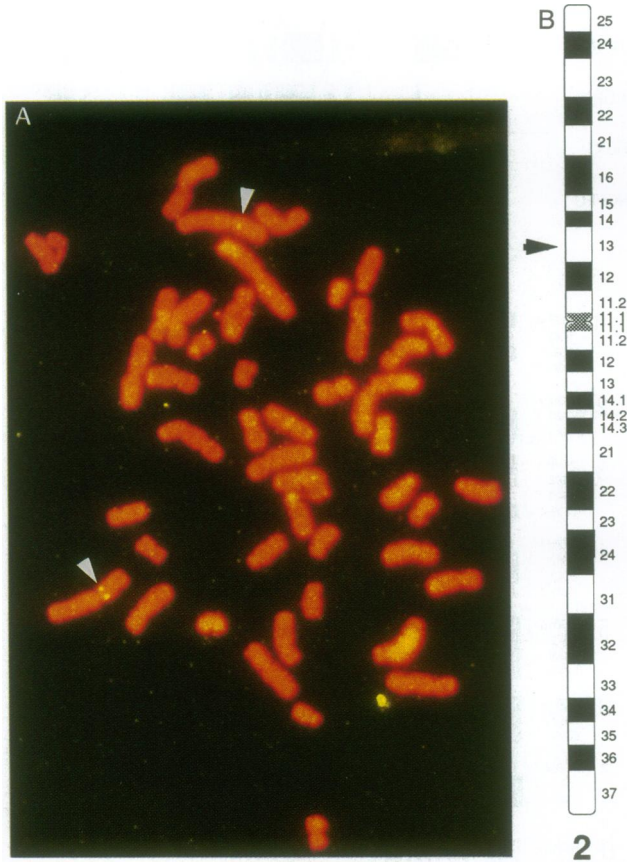

FIG. 4. Mapping of the human NGFI-C gene to chromosome 2p13 by *in situ* hybridization. (*A*) The human genomic NGFI-C clone (hλ3) hybridized to metaphase chromosomes. Arrowheads indicate hybridization to a specific site on sister chromatids of chromosome 2. Both homologues of chromosome 2 are present. (*B*) Human chromosome 2 ideogram (400-band haploid karyotype) showing localization of the NGFI-C gene to 2p13 (arrow).

phase spreads that were examined. 4,6-Diamidino-2-phenylindole dihydrochloride staining and Giemsa banding (data not shown) confirmed the signals were located at chromosome 2p13.

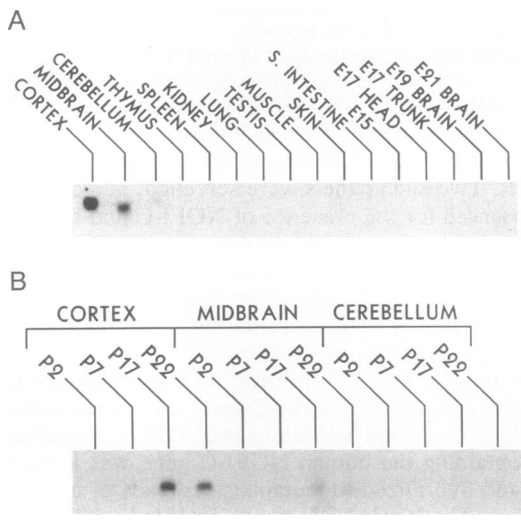

FIG. 5. Expression of NGFI-C in various rat tissues. S1 nuclease protection analysis was performed on 50 μg of total RNA isolated from the indicated adult or embryonic tissues or embryos (*A*) and cortex, midbrain, or cerebellum of brains from postnatal rats of the indicated ages (*B*).

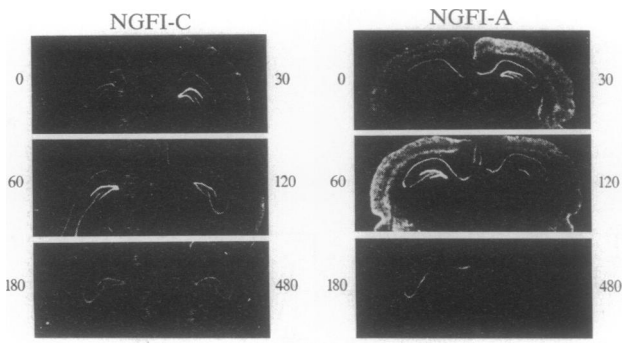

FIG. 6. Activation of NGFI-C and NGFI-A after ECT. Rats were sacrificed at the indicated times in minutes after ECT. Hemibrains from each of these six rats were embedded, sectioned, and processed together. Autoradiograms of *in situ* hybridizations performed on coronal sections taken at the level of the hippocampus and using probes specific for NGFI-C and NGFI-A are shown.

Tissue Specificity of NGFI-C Expression. We performed S1 protection analysis on RNAs isolated from a variety of adult rat tissues, and embryonic day (E) 15, E17, and E21 embryos (Fig. 5*A*). We detected NGFI-C transcripts only in adult rat brain. In addition to S1 analysis, a preliminary survey, using *in situ* hybridization, of embryos aged 11.5, 14.5, and 17.5 days indicated no embryonic expression of NGFI-C (data not shown). S1 analysis performed on cerebellum, midbrain, and cortex isolated during the early postnatal period demonstrated that NGFI-C expression was first detected by 1 week of age and increased gradually until postnatal day (P) 17, when it plateaued at levels similar to those observed in the adult (Fig. 5*B*). NGFI-C expression was highest in the cortex and almost undetectable in the cerebellum. Low levels of NGFI-C expression were also detected using the reverse transcription/PCR (17) in portions of the peripheral nervous system, including the dorsal root and superior cervical ganglia and sciatic nerve (data not shown).

NGFI-C Expression Is Activated in the Dentate Gyrus After ECT. NGFI-C (7), like many other early-response genes that encode transcription factors (e.g., NGFI-A, NGFI-B, and c-*fos*), is rapidly activated in rat brain after seizure (18). To more precisely localize the areas of the brain that express NGFI-C after seizure, *in situ* hybridization was performed on coronal brain sections harvested at various times after ECT (Fig. 6). These studies revealed that NGFI-C mRNA levels were markedly increased within 30 min after ECT and returned to basal levels within 3 hr ($n = 3$). As depicted in Fig. 6, a prominent increase in NGFI-C mRNA occurs in the granule cells of the dentate gyrus. This pattern of expression is similar to, but more restricted than, that observed with NGFI-A, which is also activated in the cerebral cortex. NGFI-C induction was more prolonged than NGFI-A, as high levels of NGFI-C mRNA were still present after 2 hr, but the signal detected for NGFI-A was greatly diminished at this point in the dentate gyrus.

DISCUSSION

We have cloned and sequenced the rat NGFI-C locus, an early-response gene that encodes a zinc-finger protein of the G(S)G element binding family (7). Other members of this family include NGFI-A (4), *Krox-20* (19), *Sp1* (20), *WT1* (21), and *EGR3* (6). They are related by the close homology of their DNA binding regions and, predictably, all recognize similar or identical nucleotide sequences. Four members of this gene family (NGFI-C, NGFI-A, *EGR2*, and *EGR3*) are early-response genes. Each has a similar intron–exon structure, with a single intron near the 5′ end of the coding region and a highly homologous DNA binding region. Moreover, our

analysis demonstrates five additional regions of homology that are shared among all four of these proteins, four of which are located in the amino-terminal region. The evolutionary maintenance of these subdomains suggests they have critical functions that are likely to include interactions with other proteins involved in transcription. The three other regions conserved among NGFI-A, EGR2, and EGR3, which are not found in NGFI-C, suggest that, although NGFI-C may bind to a similar or identical nucleotide recognition site, the consequences of its interaction with this sequence may be distinct.

The expression of NGFI-C is much more restricted than that of other members of this gene family, as it could only be detected in the adult rat brain using a sensitive S1 nuclease assay. In contrast, NGFI-A and *Krox-20* are expressed in numerous neural and nonneural tissues (refs. 17 and 22; M. A. Watson and J.M., unpublished observations). In contrast to other members of this gene family, which are expressed during embryogenesis (17, 23, 24), NGFI-C expression is restricted to the postnatal period, a pattern similar to NGFI-A expression in the brain and temporally correlated with the increases in myelination, dendritic elaboration, and electrical activity that take place in the postnatal rat brain. Both genes also respond to synaptic activity as demonstrated by their increased expression soon after seizure in the granule cells of the dentate gyrus. NGFI-A expression has been shown to be modified by different varieties of neuronal activity such as the circadian rhythm (25), the establishment of long-term potentiation (26), and physiologic synaptic activity (27). The expression of NGFI-C in these and other neuronal functions remains to be elucidated.

NGFI-C is located at human chromosome 2p13, which is also the location of a constitutive fragile site (*FRA2E*) (28). Associations between constitutive fragile sites and chromosomal rearrangements linked to specific neoplasms have been suggested (28). Several cases in which a chromosomal translocation [t(2;14)(p13;q32)] brought 2p13 into proximity of the heavy chain variable region, have been linked with adult B-cell chronic lymphocytic leukemia (29), malignant lymphoma (diffused mixed small and large cell) (30), and childhood chronic lymphocytic leukemia, a rare condition in this age group (31, 32). Interestingly, disregulation of another neuron-specific zinc-finger transcriptional regulator, T-cell translocated 1 gene (*Ttg-1*) (33), has been reported to be associated with a lymphoid neoplasm (34). As NGFI-C also encodes a zinc-finger transcriptional activator that is normally expressed only in neurons, it is possible that its translocation may also result in inappropriate lymphocytic expression and proliferation.

Note. While this paper was under review, the binding site for serum response factor-related proteins was described (35). Although binding sites for this factor are found in the regulatory regions of a number of early response genes, none are present in the 5′ flanking region of NGFI-C. The sequence of human NGFI-C (termed pAT133) has been published (36).

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1. Sheng, M. & Greenberg, M. E. (1990) *Neuron* **4**, 477–485.
2. Latchman, D. S. (1990) *Biochem. J.* **270**, 281–289.
3. Polyanovsky, O. L. & Stepchenko, A. G. (1990) *BioEssays* **12**, 205–210.
4. Christy, B. & Nathans, D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8737–8741.
5. Chavier, P., Vesque, C., Galliot, B., Vigneron, M., Dolle, P., Duboule, D. & Charnay, P. (1990) *EMBO J.* **9**, 1209–1218.
6. Patwardhan, S., Gashler, A., Siegel, M. G., Chang, L. C., Joseph, L. J., Shows, T. B., Le Beau, M. M. & Sukhatme, V. P. (1991) *Oncogene* **6**, 917–928.
7. Crosby, S. D., Puetz, J. J., Simburger, K. S., Fahrner, T. J. & Milbrandt, J. (1991) *Mol. Cell. Biol.* **11**, 3835–3841.
8. Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
9. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
10. Changelian, P. S., Feng, P., King, T. C. & Milbrandt, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 377–381.
11. Theune, S., Fung, J., Todd, S., Sakaguchi, A. Y. & Naylor, S. L. (1991) *Genomics* **9**, 511–516.
12. Lichter, P., Cremer, T., Borden, J., Manuelidis, L. & Ward, D. C. (1988) *Hum. Genet.* **80**, 224–234.
13. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
14. Cole, A. J., Abu-Shakra, S., Saffen, D. W., Baraban, J. M. & Worley, P. F. (1990) *J. Neurochem.* **55**, 1920–1927.
15. Saffen, D. W., Cole, A. J., Worley, P. F., Christy, B. A., Ryder, K. & Baraban, J. M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7795–7799.
16. Wilson, T. E., Fahrner, T. J., Johnston, M. & Milbrandt, J. (1991) *Science* **252**, 1297–1300.
17. Watson, M. A. & Milbrandt, J. (1990) *Development* **110**, 173–183.
18. Cole, A. J., Sawsan, A., Saffen, D. W., Baraban, J. M. & Worley, P. F. (1990) *J. Neurochem.* **55**, 1920–1927.
19. Chavrier, P., Janssen-Timmen, U., Mattei, M. G., Zerial, M., Bravo, R. & Charnay, P. (1989) *Mol. Cell. Biol.* **9**, 787–797.
20. Briggs, M. R., Kadonaga, J. T., Bell, S. P. & Tjian, R. (1986) *Science* **234**, 47–52.
21. Rauscher, F. J., Morris, J. F., Tournay, O. E., Cook, D. M. & Curran, T. (1990) *Science* **250**, 1259–1262.
22. Chavrier, P., Zerial, M., Lemaire, P., Almendral, J., Bravo, R. & Charnay, P. (1988) *EMBO J.* **7**, 29–35.
23. Wilkinson, D. G., Bhatt, S., Chavrier, P., Bravo, R. & Charnay, P. (1989) *Nature (London)* **337**, 461–464.
24. Pritchard-Jones, K., Fleming, S., Davidson, D., Bickmore, W., Porteous, D., Gosden, C., Bard, J., Buckler, A., Pelletier, J., Housman, D., van Heyningen, V. & Hastie, N. (1990) *Nature (London)* **346**, 194–197.
25. Rusak, B., Robertson, H. A., Wisden, W. & Hunt, S. P. (1990) *Science* **248**, 1237–1240.
26. Wisden, W., Errington, M. L., Williams, S., Dunnett, S. B., Waters, C., Hitchcock, D., Evan, G., Bliss, T. V. P. & Hunt, S. P. (1990) *Neuron* **4**, 603–614.
27. Worley, P. F., Cole, A. J., Murphy, T. H., Christy, B. A., Nakabeppu, Y. & Baraban, J. M. (1990) *Cold Spring Harbor Symp. Quant. Biol.* **55**, 213–223.
28. Yunis, J. J. & Soreng, A. L. (1984) *Science* **226**, 1199–1204.
29. Ueshima, Y., Bird, M. L., Vardiman, J. W. & Rowley, J. D. (1985) *Int. J. Cancer* **36**, 287–290.
30. Nishida, K., Taniwaki, M., Misawa, S. & Abe, T. (1989) *Cancer Res.* **49**, 1275–1281.
31. Fell, H. P., Smith, R. G. & Tucker, P. W. (1986) *Science* **232**, 491–494.
32. Watson, M. S., Land, V. J., Carroll, A. J., Pullen, J., Borowitz, M. J., Link, M. P., Amylon, M. & Behm, F. G. (1992) *Cancer Genet. Cytogenet.*, in press.
33. Greenberg, J. M., Boehm, T., Sofroniew, M. V., Keynes, R. J., Barton, S. C., Norris, M. L., Surani, M. A., Spillantini, M. G. & Rabbitts, T. H. (1990) *Nature (London)* **344**, 158–160.
34. McGuire, E. A., Hockett, R. D., Pollock, K. M., Bartholdi, M. F., O'Brien, S. J. & Korsmeyer, S. J. (1989) *Mol. Cell. Biol.* **9**, 2124–2132.
35. Pollock, R. & Treisman, R. (1991) *Genes Dev.* **5**, 2327–2341.
36. Muller, H.-J., Skerka, C., Bialonski, A. & Zipfel, P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10079–10083.
37. Yunis, J. J. (1976) *Science* **191**, 1268–1270.