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Identification of a neural-specific cDNA, NPDC-1, able to down-regulate cell proliferation and to suppress transformation

(differentiation/neural cells/regulatory genes/transcription factors)

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ABSTRACT **Immortalized neural precursor cell lines carrying the polyoma large tumor (T) gene have been shown previously to retain a clear-cut contact inhibition of growth and to differentiate *in vitro*. In the present study, we have identified and isolated cDNA clones corresponding to RNA expressed preferentially when these cells reach confluence. One of them, NPDC-1, is expressed specifically in the nervous system. The transfection of dividing cells with a NPDC-1 expression vector results in the inhibition of cell proliferation. In addition, the stable introduction of NPDC-1 into transformed cells, even of nonneural origin, leads to the suppression of transformed characteristics.**

The identification of transcriptional factors has improved our knowledge of mechanisms underlying mammalian development and cell-lineage determination (for reviews, see refs. 1–3). However, most of the molecular regulations involved in neural cell commitment and differentiation remain to be elucidated. As we previously discussed (4), this is partly due to the difficulty of establishing permanent neural cell lines that retain the differentiation properties of their normal cell counterparts. Indeed, in the case of muscle cell differentiation, permanent cell lines have been successfully used for identifying regulatory genes (5–7).

Our present purpose has been to identify genes that may be involved in the control of neural cell proliferation and differentiation. The criteria for selecting the sequences to be used as probes were both their preferential expression in neural precursor cells reaching confluence and their ability to encode amino acid stretches yielding helix–loop–helix (HLH) domains. Thus, cDNA from CLT.T.1.1 cells, which are clearly contact-inhibited (8), was amplified by the polymerase chain reaction (PCR) using oligonucleotides derived from HLH sequences and designed to minimize the amplification of already known sequences. Amplified probes that detected mRNA that was preferentially expressed when the cells became confluent were used to screen a mouse embryo brain cDNA library. The study of the isolated clones led to the identification of a gene selectively expressed in neural cells that become growth-arrested and differentiating; we have designated this gene and its products *NPDC-1* (for neural proliferation, differentiation, and control).§ The growth properties of cell lines stably transfected with NPDC-1 cDNA suggest that NPDC-1 might have an important function in the regulation of cell division and the prevention of transformation.

MATERIALS AND METHODS

Cell Lines. CLT.T.1.1, Str.SVLT.3.8, Mes.3.1 neural precursor cell lines (8–10) were grown in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal calf serum.

PCR Amplification of RNA and Cloning of Probes. Reverse PCR was carried out as described (11), with primers derived from Id and emc helix 1 (6, 12) and hairy helix 2 (13). Sense primers (with a *Bam*HI site, underlined below) were an equimolecular mixture of oligonucleotide A (5′-AACCGGATCCAAGITCAAGGAGGCGGIGCAC-3′) and oligonucleotide A modified at nucleotide 14 (C) or 24 (T) or 30 (C). Antisense primers (with an *Eco*RI site) were oligonucleotide B (5′-AACCGAATTCCAGAITCTICGCCCGGTCAAG-3′) and oligonucleotide B modified at nucleotide 17 (G) or 24 (T) or 25 (T). The PCR products were cloned into pBS(+) plasmid.

Isolation and Analysis of cDNA Clones. The cloned PCR products that revealed a differential expression were used as probes to screen a 20-day mouse embryo brain cDNA library, made in phage λgt10. After isolation of the clones, *Eco*RI inserts were subcloned into pKS II(−) plasmid, and both strands were sequenced by the dideoxynucleotide method.

Amplification and Cloning of the cDNA 5′ End. The PCR conditions were adapted from the method of amplification of cDNA ends (14). Postnatal mouse brain RNA was reverse-transcribed from primer 3 (antisense to NPDC-1 nucleotides 491–508), and a poly(dA) tail was added. Three PCR cycles (92°–52°–72°C) were performed with an oligo(dT)$_{17}$-primer adaptator, including *Xho* I–*Sal* I–*Cla* I sites, and with primer 2 (antisense to NPDC-1 nucleotides 259–275). Then, 35 cycles were achieved with the adaptor without oligo(dT) and with primer 1 (antisense to NPDC-1 nucleotides 73–90). After end-filling, the products were cleaved at the *Sal* I site and cloned into pKS II(−) plasmid.

RNA (Northern) Blot Hybridizations. After electrophoresis in 1.4% agarose/formaldehyde gel and transfer onto GeneScreen filters, cytoplasmic RNA was hybridized with the indicated probes under stringent conditions (15).

NPDC-1 Transfection. Vector pJC-NPDC-1 was constructed by fusing the 5′ end of NPDC-1 (*Sal* I–*Eco*RI fragment of pNPDC-1) with the *Acc* I–*Hin*dIII fragment of JC virus GS/B strain promoter (16). Mes.3.1 or NIH 3T6 cells were seeded at 10^4 cells per cm^2 and transfected as described (17) with 0.5 μg of pJC-NPDC-1 DNA or, for controls, with pJC-nls*lacZ*—i.e., the JC virus GS/B promoter fused to nls*lacZ* sequence from L7RHβgal (18); 48 hr later, 7.4 kBq of [^{3}H]thymidine (74 GBq/mmol) per ml was added for 16 hr and then chased with 10 μM thymidine. Digoxygenin-11-rUTP-labeled RNA probes were synthetized from pNPDC-1 with phage T3 or T7 RNA polymerase. The probe was hybridized as described (19) and revealed with anti-digoxigenin antibodies conjugated to alkaline phosphatase.

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Abbreviations: HLH, helix–loop–helix; ORF, open reading frame.

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§The NPDC-1 cDNA sequence has been registered in the GenBank and EMBL data bases (accession nos. L03814 and X67209, respectively).

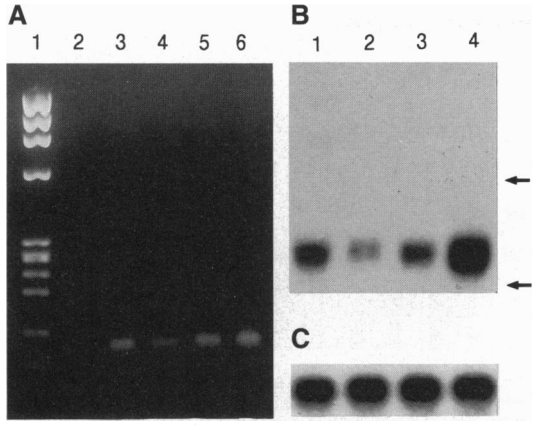

FIG. 1. Differentially expressed RNA in the CLT.T.1.1 cell line. (*A*) The cDNA from CLT.T.1.1 cells was PCR-amplified with the combination of primers described in *Materials and Methods*, and the products were analyzed by gel electrophoresis. Lanes: 1, *Hae* III fragments of ΦX 174 DNA (1353, 1078, 872, 603, 310, 281–271, 234, 194, 118, and 72 bp); 2–6, control without cDNA (lane 2), cDNA from adult mouse brain (lane 3), cDNA from exponentially growing cells (lane 4), cDNA from subconfluent cells (lane 5), and cDNA from growth-arrested cells maintained at confluence for 1 week (lane 6). (*B*) Northern blot of cytoplasmic RNA (10 μg per lane) from CLT.T.1.1 cells. The 100-bp PCR product shown in *A* was used as a probe. Lanes 1: adult mouse brain; 2–4, exponentially growing cells (lane 2), subconfluent cells (lane 3), and confluent cells (lane 4). Arrows indicate the position of RNA markers of 2.4 and 1.4 kb. (*C*) Control hybridizations with the pIL7 probe for the growth-independent cytochrome oxydase subunit II (21).

To obtain stable transfectants, 5×10^5 cells were transfected with 2.5 μg of pY3 DNA conferring hygromycin resistance (20) and 25 μg of pJC-NPDC-1 DNA (or pJC-nls*lacZ* for controls). Transfectants were selected with 200 μg of hygromycin per ml and were cloned as described (4).

RESULTS

Differential Expression of mRNA in CLT.T.1.1 Cells. At first, RNA from either proliferating or growth-arrested and differentiating CLT.T.1.1 cells (8) was reverse-transcribed and PCR-amplified with oligonucleotides A and B (see *Materials and Methods*) as primers. The PCR pattern showed a 100-bp product (Fig. 1*A*), which was cloned and used as a probe in Northern hybridizations. This revealed a 1.5-kb mRNA expressed preferentially when the cells became growth-arrested (Fig. 1*B*). The subsequent sequence determination of NPDC-1 cDNA indicated that the probe hybridized with the region from nucleotide 333 to nucleotide 400 (Fig. 2).

Cloning of the NPDC-1 cDNA. With the above-mentioned probe, we screened a 20-day-old mouse embryo brain cDNA library. An insert of 1.3 kb (NPDC-1a) included an open reading frame (ORF) of 966 nucleotides (nucleotides 38–1004 in Fig. 2) that lacked an initiation codon. The sequence was extended towards the 5′ end of NPDC-1 by PCR amplification of the cDNA end (14). After cloning of the products, 15 clones carried inserts overlapping NPDC-1a, as illustrated for NPDC-11 (Fig. 3). To obtain the complete coding sequence, NPDC-1a and NPDC-11 were cleaved at their common *Sac* II restriction site (nucleotides 36–41 in Fig. 3) and fused together. The final plasmid (pNPDC-1) carried an insert of 1348 nucleotides with an ORF of 996 nucleotides (Fig. 2) starting at a consensus initiation sequence (22). Primer extension experiments (not shown) indicated that the cap site of NPDC-1 mRNA was located 22 nucleotides upstream of the 5′ end of NPDC-1 cDNA.

NPDC-1 Does Not Belong to an Already Known Family of Regulatory Factors. The comparison of the NPDC-1 sequence with those indexed in EMBL, GenBank, NBRF/PIR and Swissprot libraries indicated that NPDC-1 does not correspond to already known genes or proteins. The analysis of the concordances between four different predictive programs for secondary structures (23–26) revealed one domain (amino acids 95–132) with two helical segments separated by a non-helical stretch (Fig. 4). The sequence from amino acid 95 to residue 113 presents a short homology (50% or 55% if conservative changes are considered) with the Fos leucine zipper (27) and GPE1-BP helical region (28) but without a heptad repeat. The sequence from amino acid 123 to residue 132 shows some homology (50% or 80%) with the Jun leucine zipper (29, 30) and a slight one with hairy helix 2 (13). How-

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   1                                                                                                                GCTGAAGC
   9  ATGGCGACGCCCGTCCCTCCGCCTTCCCCG CGGCACCTGCGGCTGCTACGGCTGCTGCTC TCCGGCCTCATCCTGGGAGCGGCCCTGAAT
   1   M  A  T  P  V  P  P  P  S  P   R  H  L  R  L  L  R  L  L  L   S  G  L  I  L  G  A  A  L  N

  99  GGTGCCACCGCCCGCCGCCCGGATGCTACC ACCTGTCCTGGAAGCCTGGATTGTGCACTG AAGAGGCGGGCAAAATGCCCACCAGGGGCA
  31   G  A  T  A  R  R  P  D  A  T   T  C  P  G  S  L  D  C  A  L   K  R  R  A  K  C  P  P  G  A

 189  CATGCCTGTGGACCATGCCTTCAGTCCTTC CAGGAAGACCAGCGAGGGTTCTGTGTGCCA AGGAAACACCTATCTTCTGGGGAGGGCCTG
  61   H  A  C  G  P  C  L  Q  S  F   Q  E  D  Q  R  G  F  C  V  P   R  K  H  L  S  S  G  E  G  L

 279  CCCCAGCCCAGACTGGAAGAGGAGATCGAT TCTCTGGCCCAGGAACTGGCACTGAAGGAG AAGGAGGCGGGGCACTCAAGGCTGACCGCA
  91   P  Q  P  R  L  E  E  E  I  D   S  L  A  Q  E  L  A  L  K  E   K  E  A  G  H  S  R  L  T  A

 369  CAGCCCTTGCTTGAGCGTGCGCAGAAGCTC CTGGAGCCTGCAGCTACCCTGGGATTCTCA CAGTGGGGTCAACGACTGGAGCCCGGGCTC
 121   Q  P  L  L  E  R  A  Q  K  L   L  E  P  A  A  T  L  G  F  S   Q  W  G  Q  R  L  E  P  G  L

 459  CCCTCTACTCATGGAACCTCTTCACCTATA CCCCATACTTCCTTGAGTTCCCGAGCATCC TCTGGACCTGTGCAGATGTCCCCGCTGGAG
 151   P  S  T  H  G  T  S  S  P  I   P  H  T  S  L  S  S  R  A  S   S  G  P  V  Q  M  S  P  L  E

 549  CCCCAGGGAAGGCATGGGAATGGCCTCACC CTTGTGCTGATCCTGGCTTTCTGCTTAGCA AGCTCAGCCGCCCTGGCTGTGGCAGCCCTC
 181   P  Q  G  R  H  G  N  G  L  T   L  V  L  I  L  A  F  C  L  A   S  S  A  A  L  A  V  A  A  L

 639  TGCTGGTGTAGGCTTCAGCGAGAGATCCGC CTGACTCAGAAGGCTGACTATGCTGCCACA GCTAAGGGGCCCACTTCACCTTCAACACCA
 211   C  W  C  R  L  Q  R  E  I  R   L  T  Q  K  A  D  Y  A  A  T   A  K  G  P  T  S  P  S  T  P

 729  CGAATCTCGCCTGGGGATCAGCGGCTGGCA CACAGTGCTGAGATGTATCATTATCAGCAC CAGAGACAGCAGATGCTGTGCCTGGAGCGG
 241   R  I  S  P  G  D  Q  R  L  A   H  S  A  E  M  Y  H  Y  Q  H   Q  R  Q  Q  M  L  C  L  E  R

 819  CATAAGGAACCTCCAAAGGAACTAGAGTCA GCTTCCTCCGATGAGGAAAATGAAGATGGC GACTTCACTGTGTATGAATGCCCAGGCCTG
 271   H  K  E  P  P  K  E  L  E  S   A  S  S  D  E  E  N  E  D  G   D  F  T  V  Y  E  C  P  G  L

 909  GCGCCAACAGGGGAAATGGAGGTGCGCAAC CCACTGTTTGACCATTCCACGCTGTCAGCA CCAGTGCCTGGTCCCCATTCCTTGCCGCCG
 301   A  P  T  G  E  M  E  V  R  N   P  L  F  D  H  S  T  L  S  A   P  V  P  G  P  H  S  L  P  P

 999  CTGCAGTGACTCCTAGGCCCACATGCCTGC AGCCCACAGCCCACCAGTGGGGACGAGCGA GTCCTGGTGTTCCCCATGGAAAAGAGCAGC
 331   L  Q  *

1089  ATTGTGACCCCCGCATGCTTTTGGCAGGAG GGAGGCGCCACCCCTGGACTCCCTTGCCAG GGAGATTCTCCGCCTTATGTGGGAGATCTA
1179  GCATCTGCTTGCACATTCTCTGTCTGGTAT GAACCCTCAAACTGGAGATCCAAGTCCAGG AACCCTAGAGCCCAGGCATGGATAGAGAGT
1269  TTTGGAGACAAAAGCCAATTAAAGTTTTTT TCAAACCTGTGAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA
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FIG. 2. Nucleotide and deduced amino acid sequence of NPDC-1. The ATG codon (nucleotides 9–11) is preceded by a TGA stop codon two triplets upstream. The coding sequence ends at nucleotide 1004 followed by TGA. A classical polyadenylylation signal is underlined.

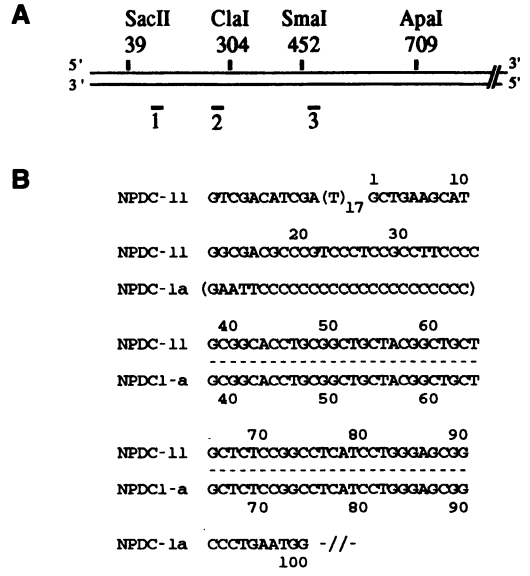

FIG. 3. Cloning of the 5′ region of NPDC-1. (*A*) Strategy of extension of the 5′ end. Horizontal bars show the position of the antisense primers. After cloning, the sequence of the amplified fragments was determined as illustrated for NPDC-11. (*B*) Alignment of NPDC-11 and NPDC-1a. Nucleotides are numbered as in Fig. 2. Thus, NPDC-11 and NPDC-1a inserts start at nucleotides 1 and 38, respectively. NPDC-11 ends at nucleotide 90. In parentheses are oligonucleotide tails preceded by the restriction sites used for the initial cloning.

ever, the homologies are too short to classify NPDC-1 in the HLH or leucine-zipper protein families. This analysis suggested also the existence of another domain (amino acids 192–229) with two helical segments separated by a nonhelical stretch but without homology with other transcriptional factors.

These two domains are separated by a Pro/Ser/Thr-rich region from amino acids 151 to 173. Another stretch of potential phosphorylation sites is found between amino acids 234 and 244.

Neural Specificity of NPDC-1 mRNA Expression. Northern blots and reverse-PCR amplification of RNA from different tissues, primary cultures, and cell lines showed that NPDC-1 RNA is expressed in brain, while it is undetectable in liver, heart, skeletal muscle, spleen (Fig. 5 *A* and *B*), pancreas, pituitary, and adrenal glands (Fig. 5*B*). This preferential expression in the nervous system was confirmed by *in situ* hybridization experiments (data not shown; E.D., E.G., C.E., and P.R., unpublished results). While NPDC-1 was hardly detect-

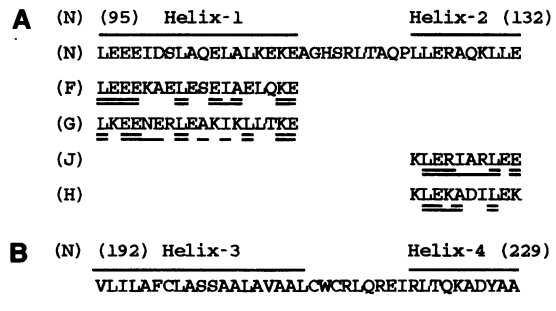

FIG. 4. Predicted helical regions of NPDC-1. (*A*) Helices 1 and 2 of NPDC-1 (N) are indicated by a bar above the sequence and are aligned with sequences 190–207 of Fos B (F), 99–114 of GPE1-BP (G), 276–285 of c-Jun (J), and 71–80 of Hairy (H). Identical amino acids or conservative changes with NPDC-1 residues are indicated with a double or a single underline, respectively. (*B*) Helices 3 and 4 of NPDC-1 are indicated by a bar above the sequence.

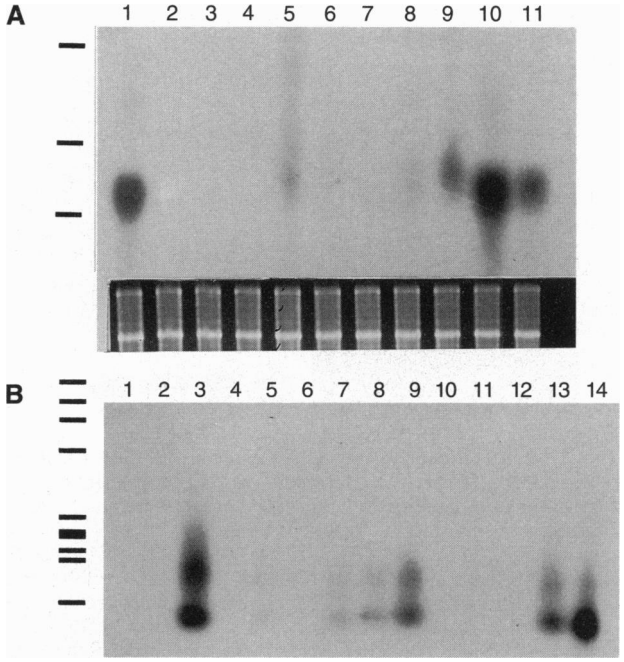

FIG. 5. Selective expression of NPDC-1 RNA. (*A*) Northern hybridizations. After gel electrophoresis and transfer, total RNA (10 μg per lane) from different tissues and cells was hybridized with ^{32}P-labelled NPDC-1 cDNA. Lanes: 1–7, postnatal brain, liver, heart, hind limb extensor muscle, kidney, spleen, and adrenal gland, respectively; 8 and 9, primary astrocytes maintained 7 days (still dividing) and 15 days (confluent) in culture, respectively; 10 and 11, primary striatal postmitotic neurons 7 and 15 days after seeding, respectively, in conditions limiting the proportion of astrocytes under 5% during the first week in culture and as described (4). Bars indicate the migration of the markers (4.4, 2.4, and 1.4 kb). (*A Inset*) Staining of 28S and 18S RNAs with ethidium bromide. (*B*) Cytoplasmic RNA was amplified by reverse-PCR-amplification with the primers described in *Materials and Methods*. After gel electrophoresis, the amplified cDNA was analyzed with the above-mentioned probe. Bars indicate the position of the markers (1353, 1078, 872, 603, 310, 281, 271, 234, 194, and 118 bp). Lanes: 1, markers; 2, control without reverse transcription; 3–6, cDNA from mouse brain, liver, heart, and hind limb extensor muscle, respectively; 7–9, cDNA from exponentially growing, subconfluent, and differentiating CLT.T.1.1 cells maintained at confluence for 1 week, respectively (8); 10–12, cDNA from mouse pancreas, pituitary gland, and adrenal gland, respectively; 13 and 14, cDNA from the bipotent neural precursor cell line Str.SVLT.3.8 differentiating towards neurons in defined N2 medium or towards astrocytes in standard medium (9), respectively.

able in dividing astrocytes, it was expressed in growth-arrested primary astrocytes and neurons (Fig. 5*A*, lanes 7–10). Results in lanes 7–9, 13, and 14 of Fig. 5*B* confirmed also that NPDC-1 expression in CLT.T.1.1 and Str.SVLT.1.3 lines increased as the cells approached confluence and increased dramatically when they were growth-arrested and began to differentiate (8, 9).

Anti-Proliferative Effect of NPDC-1. The effect of NPDC-1 ectopic expression on cell proliferation was examined. First, after transfection of exponentially growing Mes.3.1 cells with pJC-NPDC-1, DNA synthesis was inhibited in the cells expressing NPDC-1, whereas control-transfected cells were [^{3}H]thymidine-positive and NPDC-1-negative (Fig. 6).

Then, NIH 3T6 and C6 glioma cells (31) were stably transfected with pJC-NPDC-1 and pY3 plasmid conferring hygromycin resistance. At first, we observed that the frequency of drug-resistant clones (10^{-5}) was 30-fold lower than in control experiments (cotransfections with pY3 and pJC-nls*lacZ*), as if inappropriate or overexpression (Fig. 7*A*) of the introduced NPDC-1 impeded cell proliferation.

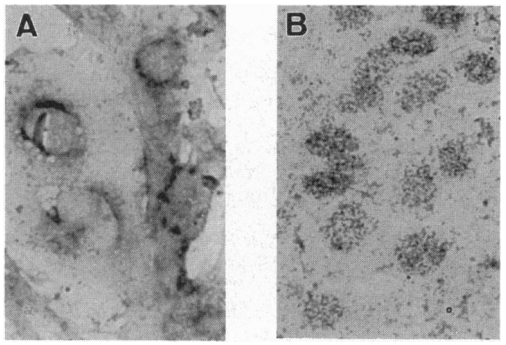

FIG. 6. Effect of NPDC-1 expression on DNA synthesis. Mes.3.1 cells were transfected with pJC-NPDC-1 (*A*) or with pJC-nls*lacZ* for control (*B*); 48 hr later, [^{3}H]thymidine was added for 16 hr. *In situ* hybridization was performed with NPDC-1 antisense digoxygenin RNA probe. Then the cells were processed for autoradiography and immunodetection of the hybridized probe. (×400.)

This hypothesis was strengthened by the other results illustrated in Fig. 7. Indeed, the stable transfections resulted in important modifications of the growth properties of the cells. The doubling time of the NIH 3T6-NPDC-1 lines ranged from 18 to 20 hr versus 12 hr for the control-transfected cells. Regarding the C6-NPDC-1 clones, the generation time ranged from 36 to 48 hr versus 23 hr for the control cells. The saturation density of the NPDC-1-transfected lines was significantly decreased (2- to 4-fold, depending on the line) as compared with the controls (Fig. 7*B*). We observed also that the addition of NPDC-1 antisense oligonucleotides (nucleotides 2–25) to the transfected lines restored the generation time of the control cells and led to an increased [^{3}H]thymidine incorporation, which was also observed for CLT.T.1.1 cells expressing endogenous NPDC-1 (Fig. 7*C*). At every point along the growth curves, the proportion of trypan blue-stained or floating cells was less than 2%, while the proportion of cells with a chromatin condensation suggestive of apoptosis was below 0.1%. Furthermore, the stable transfection of NIH 3T6 and C6 cells with NPDC-1 led to the suppression of the capacity of the cells to grow in semisolid medium (Fig. 7 *D* and *E*).

DISCUSSION

In this study, we have identified a cDNA encoding a protein that down regulates cell proliferation. The strategy has been to investigate RNAs that are increasingly expressed when CLT.T.1.1 cells reach confluence and that are susceptible of encoding proteins with HLH-like domains. Thus, for reverse-PCR amplification of RNA, we used primers derived from ld, emc, and hairy (6, 12, 13) but different enough to minimize the amplification of classical sequences. This yielded an amplified band of 100 bp, which revealed RNA expressed preferentially at confluence. Then this amplified cDNA was used as a probe to screen a mouse embryo brain cDNA library. The isolation of the clones hybridizing with this probe and the extension of the inserts by PCR amplification of cDNA ends (14) has led to the whole coding sequence of NPDC-1—i.e., an ORF of 996 nucleotides starting at a consensus initiation sequence (22).

The sequence analysis has shown that the encoded protein is different from already known proteins. The concordances between several programs (resulting from different and complementary predictive considerations) for secondary structures (23–26) have revealed two regions reminiscent of HLH domains. However, in short homologies with some HLH or leucine-zipper proteins, the canonical residues or repeats of these families are not present, indicating that NPDC-1 is not a member of a classical protein family.

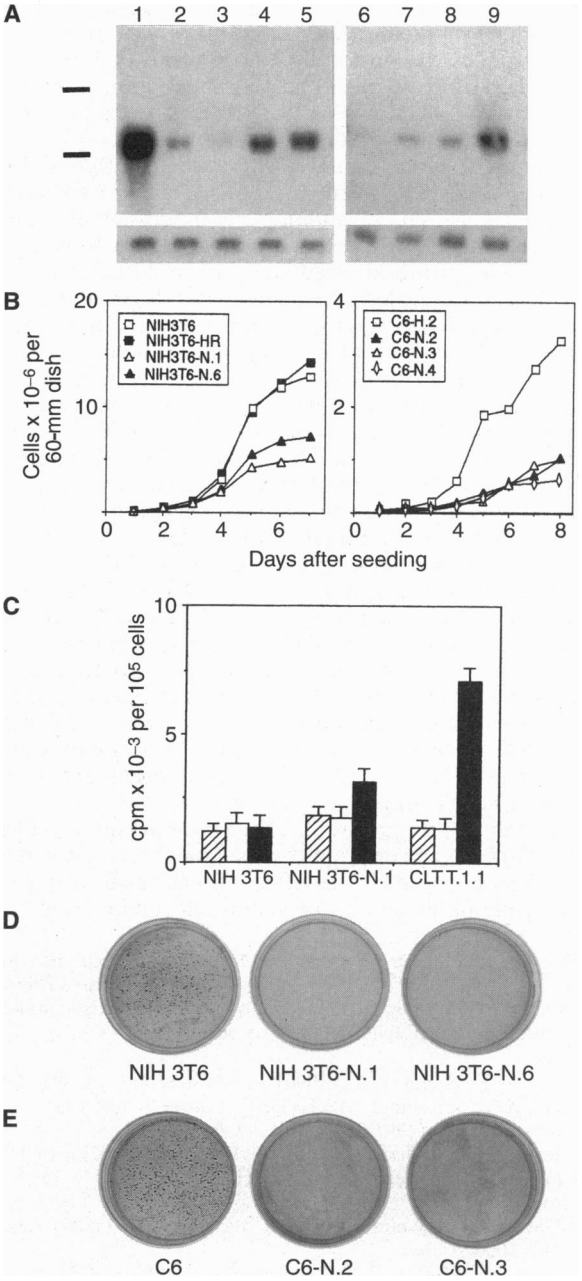

FIG. 7. Growth properties of NPDC-1 stable transfectants. (*A*) NPDC-1 RNA expression in different cell lines. Northern hybridizations were performed as described in Fig. 5*A*. Lanes: 1–5, RNA from confluent CLT.T.1.1, NIH 3T6, NIH 3T6-HR (hygromycin-resistant clone) NIH 3T6-N.1, and NIH 3T6-N.6 (independent NPDC-1-transfected clones) cells, respectively; 6–9, C6-HR (hygromycin-resistant C6 clone), C6-N2, C6-N3, and C6-N4 (NPDC-1-transfected clones) cells, respectively. Bars indicate the migration of markers of 2.4 and 1.4 kb. (*A Inset*) Control hybridizations with the pIL7 probe for the growth-independent cytochrome oxydase subunit II (21). (*B*) Growth kinetics of NIH 3T6, C6 cells, and transfected clones in standard medium. (*C*) Effect of NPDC-1 antisense oligonucleotides on [^{3}H]thymidine incorporation. ▨, Untreated cells; □, cells treated for 48 hr with 50 μM sense oligonucleotides; ■ cells subjected to 50 μM antisense oligonucleotides. Values are the means ± SEM of three independent experiments. (*D* and *E*) Suppression of growth in the absence of anchorage. Cells (5×10^4) were grown for 3 weeks in standard medium containing 0.3% agarose over an underlayer of 0.5% agarose.

NPDC-1 displays two striking Pro/Ser/Thr-rich clusters. One includes 11 serine or threonine residues and 4 proline residues within the 22 amino acids from 151 to 173. The other one

corresponds to the sequence Pro-Thr-Ser-Pro-Ser-Thr-Pro-Arg-Ile-Ser-Pro, (amino acids 234–244), which presents three consensus sites recognized by mitogen-activated protein kinases (32, 33). This suggests that phosphorylation events might be involved in the function of NPDC-1, even though this remains to be demonstrated.

The study of NPDC-1 mRNA expression in different tissues shows that the NPDC-1 gene is expressed almost exclusively in the nervous system. In addition, its expression in primary neural cells and in cell lines is dramatically increased when the cells become growth-arrested and start to differentiate.

Evidences for a down-regulation of cell proliferation by NPDC-1 have arisen from the study of the growth properties of the NIH 3T6 and C6 lines stably transfected with NPDC-1. Indeed, their doubling time is 1.5- to 2-fold (depending on the line) higher than that of parental and control-transfected cells. This increase of the generation time is actually due to the expression of NPDC-1, since the addition of NPDC-1 antisense oligonucleotides to the transfectants led to the recovery of the parental doubling time and to an increase (up to 3 fold) in [^{3}H]thymidine incorporation. This last effect was also observed with lines expressing endogenous NPDC-1.

In addition, the saturation density of NPDC-1 stably transfected lines is lower than that of control lines by a factor of 2–4. Since the proportion of dead cells was always less than 2%, it is unlikely that this was due to cell death. Furthermore, NIH 3T6-NPDC-1 and C6-NPDC-1 lines are no longer able to form multilayers or to grow in semisolid medium. These results show that NPDC-1 stable transfection leads to the suppression of transformation and to the recovery of a normal growth control, even in cells of nonneural origin.

Even though the precise mechanism of action of NPDC-1 remains to be elucidated, our results show that NPDC-1 is able to suppress oncogenic transformation and might play an important role in the control of neural cell proliferation.

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