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Rat TAFII$_{31}$ Gene Is Induced upon Programmed Cell Death in Differentiated PC12 Cells Deprived of NGF[1]

Tomokazu Aoki,*,†,[2] Tatsuro Koike,‡ Toru Nakano,* Keiichi Shibahara,* Hiroyuki Nishimura,* Haruhiko Kikuchi,† and Tasuku Honjo*

**Department of Medical Chemistry, †Department of Neurosurgery, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan; and ‡Graduate Program in Biological Science, Hokkaido University, Sapporo, Japan*

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Typical programmed cell death (PCD) requires de novo macromolecular synthesis and shares common morphological changes referred to as apoptosis. To elucidate the molecular mechanism of apoptosis, we isolated cDNA clones that are induced in differentiated PC12 cells deprived of NGF by differential display method. Among such clones, homology searches revealed that the one clone encodes the rat TATA-binding-protein-associated factor TAFII$_{31}$, a component of TFIID, and a transcriptional coactivator of the p53 protein. Northern analysis of various organs in human showed one band in heart, brain, skeletal muscle and pancreas, whose size is ~1.1 kb which identical to that of human TAFII$_{31}$ mRNA, although the size of rat human TAFII$_{31}$ mRNA is ~2.7 kb. The deduced amino acid sequence of the rat TAFII$_{31}$ was 77% identical to that of the human TAFII$_{31}$. Northern analysis of various organs in adult mice showed that expression levels of TAFII$_{31}$ mRNA were strong in heart but weak in spleen, although this gene is ubiquitously expressed.

Development and homeostasis of most multicellular organisms are critically dependent on mitosis, differentiation, and cell death. For example, in vertebrates approximately half of the neurons generated during neurogenesis are eliminated by cell death. This elimination serves to match the number of inervating neurons to the size of target (1, 2, 3).

It is important to set up an *in vitro* neuronal cell death system that consists of a homogeneous neuronal population available in large quantities, to get such gene products involved in cell death.

In the PCD using a subline of PC12 cells, PC12 (22a), whose death can be induced by NGF removal and blocked by an RNA synthesis inhibitor Actinomycin D, we found that expression of the rat TATA-binding-protein-associated factor TAFII$_{31}$ gene is induced. This protein is a component of TFIID, and a transcriptional coactivator of the p53 protein (4).

MATERIALS AND METHODS

Cell culture. Mouse NGF (2.5S) was isolated from male mouse submaxillary glands (5). Antiserum against mouse 2.5S NGF was kindly donated by E. M. Johnson, Jr. (Washington Univ., St. Louis, MO). PC12 (22a) cells were grown at 36.5°C in collagen-coated plates in RPMI 1640 medium with 5% fetal bovine serum and in 10% heat-inactivated horse serum (JRH Biosci, Lexena, KS) as described (6). The cells that had been previously treated with NGF for 2 weeks were deprived of NGF by adding goat antiserum against mouse NGF (1 %) (6). Dissociated sympathetic neurons were prepared from superior cervical ganglia of newborn Wistar rats (Koike et al., 1989). These cells were typically plated on collagen coated dishes (Costar Data Packaging Corp., Cambridge, MA). Cultures were grown for 7 days in Eagle's minimal essential medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum 20 μM fluorodeoxyuridine and 20 μM uridine to kill non-neuronal cells, and 50 ng/ml 2.5S NGF. To enforce rapid and complete NGF deprivation, polyclonal antiserum against NGF were added to the culture medium at a final concentration of 1% (5).

Differential display. The differential display was performed as described in the original work (7). Briefly, mRNA from differentiated PC12 cells and these cells deprived of NGF were reversed transcribed and then amplified by PCR using a combination of 20 different 5′ primers (arbitrary 10-mers) and four different 3′ primers (two-base anchored oligo(dT) primers) (RNA map Kit, GenHunter, Brookline, MA), following the manufacture's instructions.

RNA isolation and analysis. Total cellular RNA was isolated from cultured cells or various mouse organs by standard methods (8), and poly(A)$^+$ RNA was selected by oligotex (Takara, Co. Ltd., Japan). Approximately 20 μg of total RNA or 2 μg poly(A)$^+$ RNA was used for standard Northern blot analysis (8). Human multiple tissue Northern filter (MTN, Clontech) was purchased. A probe used for internal control was the 3′-UTR of the mouse β-actin cDNA (9).

cDNA library construction. Total cellular RNA was prepared from PC12 cells that were previously treated with NGF for 2

[1] The sequence data has been submitted to the GenBank database under the Accession No. U40188.

[2] To whom correspondence should be addressed. Fax: 06-752-9501. E-mail: tomokazu@kuhp.kyoto-u.ac.jp.

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weeks and cultured for 16 h without NGF by adding antiserum against NGF. Five μg of poly $(A)^+$ RNA was used to synthesize cDNA primed with an oligo (dT)-adapter primer using a cDNA synthesis kit (Pharmacia). The cDNA was ligated into the *Eco*RI site of λgt10. This library contained 10^6 independent clones carrying inserts of more than 1.5 kb in average with a range of 0.7-5.5 kb.

DNA sequencing. The cDNA library was plated on nylon membrane filter. Positive clones were digested by restrition enzymes, cloned into Bluescript SK (−) plasmid vectors (Stratagene). These plasmid DNA templates were analyzed using ABI 373 DNA sequencer (Applied Biosystems, ABI). The sequences were compared with nucleic acid sequences in GenBank.

RESULTS AND DISCUSSION

PC12 cells differentiated by culturing in the presence of NGF for 2 weeks. Such treatment gave rise to cultures containing flattened, attached, phase-bright cells with an extensive network of robust neuritis. Subsequent withdrawal of NGF by ant-NGF antibody to the medium, led to cell death within 48-72 h. (6). Their cell death was characterized by apoptotic changes including DNA fragmentation, shrinkage of the cell with preservation of the organelles, and blebbing in the cytoplasm (data not shown). We have confirmed that the PCD of this PC12 cells is blocked by the RNA inhibitor, Actinomycin D (10). The time at which 50% of NGF deprived PC12 cells are committed to die was within 12-16 h after NGF deprivation (10). Using this cell line, we have cloned one gene (DN7) whose expression is induced in differentiated PC12 cells deprived of NGF using differential display (see Materials and Methods). The DN7 mRNA is gradually induced during the PCD in differentiated PC12 cells deprived of NGF by Northern blot hybridization (Fig. 1). This result was examined using cloned DN7 fragment or full-length cDNA probe, five times. The cloned DN7 fragment was used to screen a cDNA library from differentiated PC12 cells deprived of NGF to obtain the full-length cDNA clone. One clone that hybridized to DN7 fragment could be isolated. The clone was sequenced, and the complete nucleotide sequence of 2477 bp which agrees with the size of mRNA estimated by Northern analysis is shown in Fig. 2. The sequence revealed that the longest open reading frame predicts a sequence of 253 amino acid from the first phase ATG coden.

The amio acid sequence of DN7 was found to be similar to that of the human TATA-binding-protein-associated factor $TAFII_{31}$ protein (4). The homology of the two sequences is 77% (Fig. 3).

Northern blot hybridization analysis using mRNA from various tissues of adult mice revealed the band (2.7 kb) of DN7 (Fig.4). Expression levels of DN7 mRNA were strong in heart but weak in spleen, although this gene is ubiquitously expressed. And, Northern blot hybridization analysis using mRNA from various tissues of adult human revealed one band (1.1 kb) (Fig. 5), which agrees with the size of human $TAFII_{31}$ mRNA reported by Lu and Levine (4), expression profiles of DN7 in mice are similar to those of human $TAFII_{31}$ gene, although the intensity of the band is weak. Therefore, we conclude that DN7 is the rat homolog of $TAFII_{31}$.

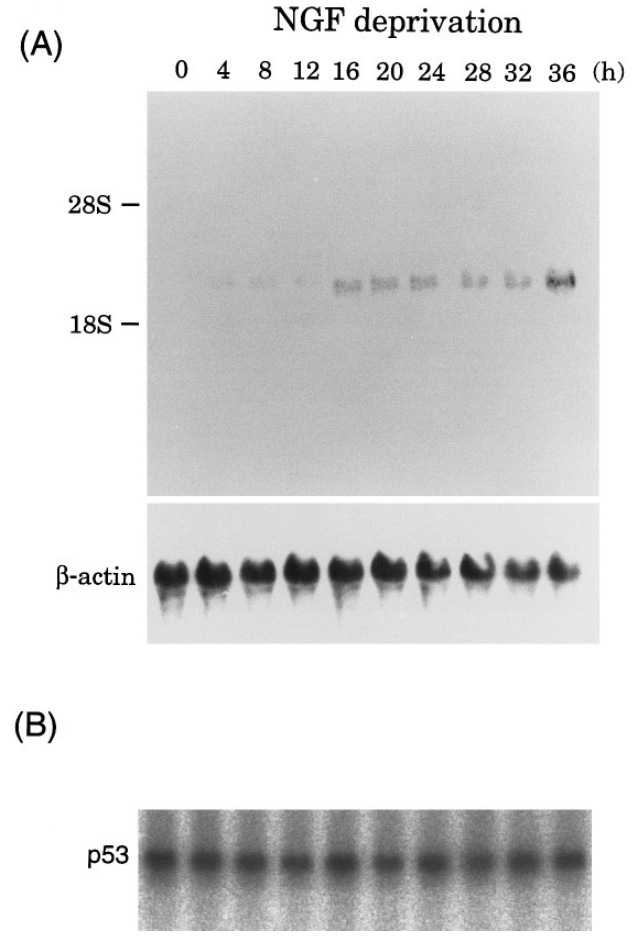

FIG. 1. (A) Northern blot analysis of DN7 mRNA in differentiated PC12 deprived of NGF. PC12 cells were maintained with NGF (50 ng/ml) for 14 days and then deprived of NGF for the indicated intervals. 20 μg of total RNA were loaded onto each lane of the gel. (B) Expression of p53 mRNA in differentiated PC12 deprived of NGF. Poly$(A)^+$ RNA (5 μg) of total RNA were loaded onto each lane of the gel.

When eukaryotic enhancer binding factors are tethered to the template DNA binding domains, appropriately exposed activation domains contact one or more subunits of the basal transcriptional apparatus (11). Many sequence-specific activators interact directly with one or more components of the basal trannscription factor IID (TFIID). TFIID is composed of the TATA binding protein (TBP) and eight or more TBP-associated factors (TAFIIs) (12). Differ-

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CGCGGCCGCTGCAAGATGGCGCCTCTCAAGAACGCTCCGAGGGATGCCTTGGTGATGGCACAGATCCTGAAGGATATGGGAATCACAGATTATGAGCCAA   100
            M  A  P  L  K  N  A  P  R  D  A  L  V  M  A  Q  I  L  K  D  M  G  I  T  D  Y  E  P  R
GGGTTATAAATCAAATGTTGGAATTTGCCTTCCGATATGTGACTACAATTCTGGATGATGCAAAAATTTATTCAAGTCACGCTAAGAAACCTACTGTGGA   200
  V  I  N  Q  M  L  E  F  A  F  R  Y  V  T  T  I  L  D  D  A  K  I  Y  S  S  H  A  K  K  P  T  V  D
TGCAGATGATGTGAGACTGGCCATCCAGTGTCGGGCTGACCAGTCTTTTACCTCTCCTCCTCCAAGAGATTTTTTACTGGATATTGCAAGGCAGAAGAAT   300
 A  D  D  V  R  L  A  I  Q  C  R  A  D  Q  S  F  T  S  P  P  P  R  D  F  L  L  D  I  A  R  Q  K  N
CAAACCCCTCTGCCGCTGATTAAACCATATGCAGGACCTAGATTGCCACCTGATAGATACTGCTTAACAGCTCCAAACTATAGGCTGAAGTCCTTAGTTA   400
Q  T  P  L  P  L  I  K  P  Y  A  G  P  R  L  P  P  D  R  Y  C  L  T  A  P  N  Y  R  L  K  S  L  V  K
AAAAGGGACCTAACCAAGGAAGACTAGTTCCTAGATTAAGTGTCGGTGCGGTCAGTAGCAGACCTACTACTCCTACTGTAGCACCCCCACAAGCAGTATC   500
  K  G  P  N  Q  G  R  L  V  P  R  L  S  V  G  A  V  S  S  R  P  T  T  P  T  V  A  P  P  Q  A  V  S
TGTCCCAAATAAGGTTGCAACTCCAGTGTCAGTGACAAGCCAAAGATTTGCCGTGCAGATTCCAACTTCTCAGTCCACGCCTGCCAAACCAGCTCCTGCA   600
 V  P  N  K  V  A  T  P  V  S  V  T  S  Q  R  F  A  V  Q  I  P  T  S  Q  S  T  P  A  K  P  A  P  A
GCGACTTCAGTCCAAAATGTTCTGATTAATCCTTCAATGATTGGGTCCACAAATATTCTCATTACCACCAACATGGTTTCCTCACAGAGCACAGCCACTG   700
A  T  S  V  Q  N  V  L  I  N  P  S  M  I  G  S  T  N  I  L  I  T  T  N  M  V  S  S  Q  S  T  A  T  D
ACTCAAACCCACTGAAGAGGAAACATGATGATGATGATGATGACGACGACGATGATGATGACAATGACACTATGTAAGGAATAAAGTCTGTTCTGTATAC   800
  S  N  P  L  K  R  K  H  D  D  D  D  D  D  D  D  D  D  D  N  D  T  M
ATTAAAATTTTCCTGTGTTTGGGCCCATATGGTGGACTCAACAGCTTAGATCTTATAAGTTTTATCTTAGAAATGTAGTAGGAATATTTTTCACATTAGT   900
TACATCGGGTAGGTTTTGTTACAATGAATATGTTTTCTCAGTAGGCTTCAGGTATACAAGCACTTGCTTCTAGACAGCTTCATTAATGTCGAGTCTTACC  1000
ATGGCAGCTCTATTTTTAGAAGCAGTTTCTGCTATGTGTCACTGACAGCGGCACTTGGATTTCAGTTCCTGGTCTCCTCTATATTAAAACAGGACTTTCT  1100
TTTGATAGTGAACTATTCACACTGTCACTTTTTTGACCAAATCTCTCAGTTTGGGAAACTTTTTTTTGTGAAACGGTGGATTTAGAAGTGTGCTAAATCCA  1200
TTTTAGAATGTATTTGGACTTTGGTCTTTTCTAATTCCCTATCTGGATCCCATTTGTTTTCTGTTCCCACCTCTCATCGCCTTAAGTCATTTGCCATTGC  1300
ACTTAAATTTAATAGACTGTGACTAGAATTTGTAATATGCCCTAGATGCCTAGCCTTCTGCAGTCCCCTTGAACCTGAGTGTCGGCATTTACTCTTCTTC  1400
ACTCGGTAACAGAGCATTTGGGTGTCAGCCCTTGCCTTTAACTCCTGTTAAGCTCTTCTCAATGACAGGCAGGTTTGCATCAGTTACATGCAGTTACCAT  1500
GCATTCCAAATTGCTAGCTTCACAGTCTTTCATGTTTTTAATTTAAGATATAACTTTTAACCTGCCTTGATAAGTAACTTACACTGGACCCAGACAAAAC  1600
CACTTAAGAGCTTGGTTTTTACCCCCTGCATTCATTGATTTCATTCAGGTCACCTTTCTAGCTTGCTGCTAAGGGTGTCATACCTAAGACCAAGCCTGAG  1700
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GTTTCCTTTTTTTTTTTTTTTCTTTTTTTTTCAGAGCTGGGGACCGAACCCAGCGCTCTACTACTGAGCTAAATCCCCACCCCCCCCTCCCAGCCATGTTT  1900
CTATACACCTTCTCCTACTTTGTTTTTATACATACCTACATGACTCATTGGAAACAACAGCAAAGCTNTCCGTCAGAGTATAAAAAGTACCATTTTGATT  2000
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TATCATAAGAGATGTGCAGTATACAACACAATGTCAGGACTTGACTGTTCTCAGGGAGGCAGAATATCAGAAAGCCTAGGTTCTAGTATTAAGTTTCATT  2300
TACCCCCAATGTGACCTTACTTTACCCCTGAGCCTTGTTCTCACTAGTAGTAGGGGAATAACATCTACCCTGCCTACCTCACAGAGATGTGNGGGTTTAA  2400
CTGGTGTGTGTGGAAGATCCAGATTGTAAAAGCCAAATAATTATTAATGTATTTGTTCCCCCCACTATTAAAAAAAA                          2477
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FIG. 2. Nucleotide sequence of DN7 cDNA and its deduced amino acid sequence.

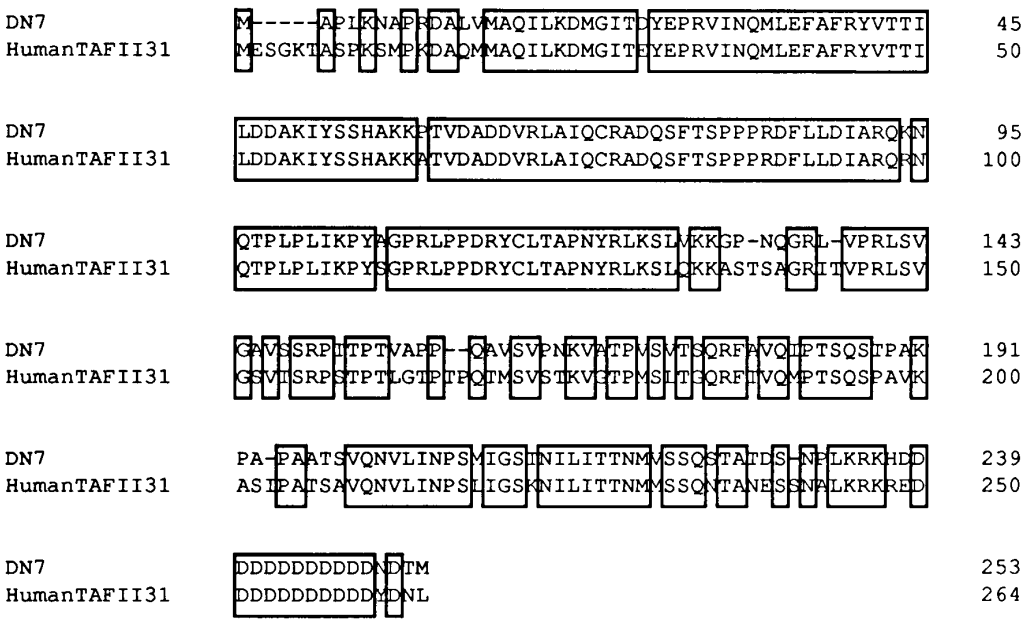

FIG. 3. Comparison of the deduced amino acid sequence of DN7 (rat $TAFII_{31}$) and human $TAFII_{31}$ (4). Conserved amino acids are circled.

ent classes of activators (such as acidic, glutamine rich, isoleucine rich) contact distinct target coactivators with in the TFIID complex to mediate transcriptional activation (13). Human $TAFII_{31}$ protein is a transcriptional coactivator of the p53 protein (4). And, similar results of experiments utilizing the TAFII40 and TAFII60 from Drosophila or fusion proteins with hTAFII31 (13). The major cellular negative regulator of p53 activity , MDM2 (14), binds to the same region of p53 protein with which hTAFII31 proteins interact (4, 15). There is a functional link between p53 and various growth-regulatory processes, including cell cycle progression (p21 / WAF1) (16, 17), DNA repair (GADD45) (18) and apoptosis (19, 20). mRNA levels of p53 in our system is unchanged, but mRNA of rat TAFII31 homolog is elevated (Fig. 1), although whether MDM2, p21, GADD45 and bax gene are induced or not is still remaining. Therefore, rat $TAFII_{31}$ homolog may play a role in PCD.

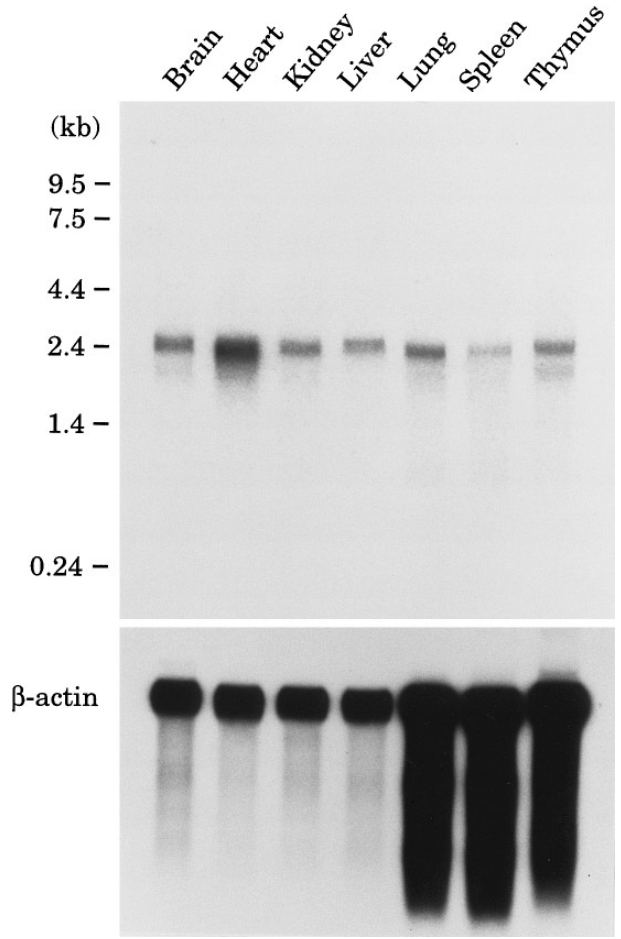

FIG. 4. Expression of DN7 mRNA in mouse adult tissues. Poly(A)$^+$ RNA (2 μg) were loaded on each lane. DN7 full-length cDNA was used as a probe.

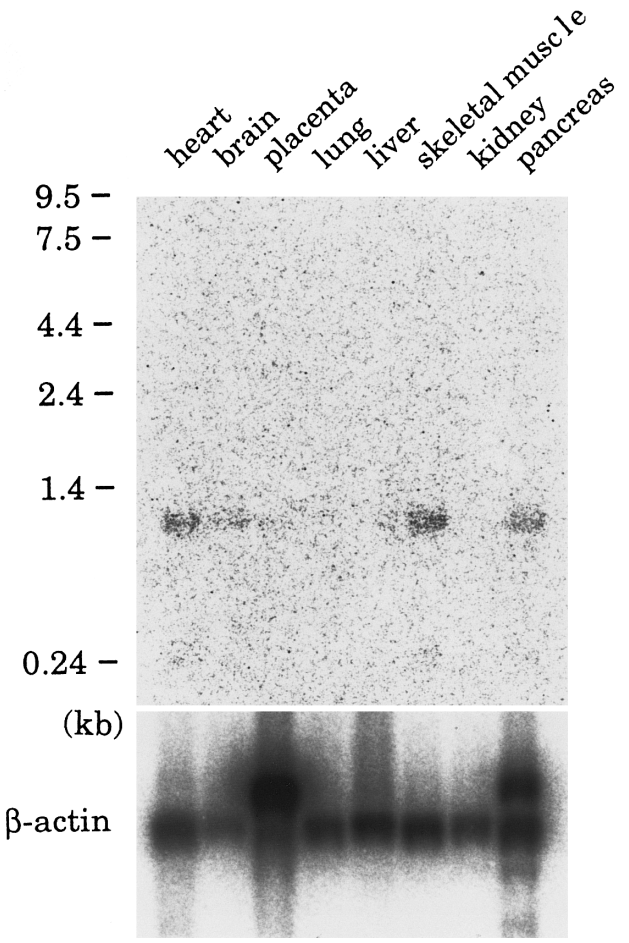

FIG. 5. Expression of DN7 mRNA in human adult tissues. Poly(A)$^+$ RNA (2 μg) were loaded on each lane. DN7 full-length cDNA was used as a probe.

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REFERENCES

1. Cowan, M. M., Fawcett, J. W., O'Leary, D. D. M., and Stanfield, B. B. (1984) *Science* **225,** 1258.
2. Martin, D. P., Schmidt, R. E., DiStefano, P. S., Lowry, O. H., Carter, J. G., and Johnson, J. E. M. (1988) *J. Cell Biol.* **106,** 829–844.
3. Oppenheim, R. W. (1991) *Annu. Rev. Neurosci.* **14,** 453–501.
4. Lu, H., and Levine, A. J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5154–5158.
5. Koike, T., Martin, D. P., and Johnson, J. E. M. (1989) *Proc. Natl. Acad. Sci. USA* **1989,** 6421–6425.
6. Koike, T. (1992) *Prog. Neuropsychopharmacol Biol. Psychiatry* **16,** 95–106.

7. Liang, P., and Pardee, A. B. (1992) *Science* **257,** 967–971.
8. Sambrook, J., Fritsch, E., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
9. Tokunaga, K., Taniguchi, H., Yoda, K., Shimizu, M., and Sakiyama, S. (1986) *Nucleic Acids Res.* **14,** 2829.
10. Aoki, T., Koike, T., Nakano, T., Shibahara, K., Kondo, S., Kikuchi, H., and Honjo, T. (1997) *J. Biochem*. **121,** 122–127.
11. Tjian, R., and Maniatis, T. (1994) *Cell* **77,** 5–8.
12. Goodrich, J. A., and Tjian, R. (1994) *Curr. Opin. Cell Biol.* **6,** 403–409.
13. Thut, C. J., Chen, J. L., Klemm, R., and Tjian, R. (1995) *Science* **267,** 100–104.
14. Momand, J., Zambetti, G. P., Olson, D. C., George, D., and Levine, A. J. (1992) *Cell* **69,** 1237–1245.
15. Lin, J., Chen, J., Elenbaas, B., and Levine, A. J. (1994) *Genes Dev.* **8,** 1235–1246.
16. El Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993) *Cell* **75,** 817–825.
17. Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993) *Cell* **75,** 805–816.
18. Smith, M. L., Chen, I. T., Zhan, Q., Bae, I., Chen, C. Y., Gilmer, T. M., Kastan, M. B., O'Connor, P. M., and Fornace, A. J., Jr. (1994) *Science* **266,** 1376–1380.
19. Oltvai, Z. N., Milliman, C. L., and Korsmeyer, S. J. (1993) *Cell* **74,** 609–619.
20. Miyashita, T., and Reed, J. C. (1995) *Cell* **80,** 293–299.