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The nerve growth factor receptor gene is at human chromosome region 17q12–17q22, distal to the chromosome 17 breakpoint in acute leukemias

(myeloid leukemia/chromosome translocation/p53/*in situ* hybridization)

KAY HUEBNER*, MASAHARU ISOBE*, MOSES CHAO†, MARK BOTHWELL‡, ALONZO H. ROSS*, JANET FINAN§, JAMES A. HOXIE¶, AMITA SEHGAL†, C. RANDY BUCK†, ANTHONY LANAHAN‡, PETER C. NOWELL§, HILARY KOPROWSKI*, AND CARLO M. CROCE*

*The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104; †Department of Cell Biology and Anatomy, Cornell University Medical College, 1300 York Avenue, New York, NY 10021; ‡Department of Molecular Biology, Princeton University, Princeton, NJ 08544; §Departments of Medicine and Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; and ¶Department of Hematology-Oncology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

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ABSTRACT **Genomic and cDNA clones for the human nerve growth factor receptor have been used in conjunction with somatic cell hybrid analysis and *in situ* hybridization to localize the nerve growth factor receptor locus to human chromosome region 17q12–q22. Additionally, part, if not all, of the nerve growth factor receptor locus is present on the translocated portion of 17q (17q21–qter) from a poorly differentiated acute leukemia in which the chromosome 17 breakpoint was indistinguishable cytogenetically from the 17 breakpoint observed in the t(15;17)(q22;q21) translocation associated with acute promyelocytic leukemia. Thus the nerve growth factor receptor locus may be closely distal to the acute promyelocytic leukemia-associated chromosome 17 breakpoint at 17q21.**

The interaction between nerve growth factor and nerve growth factor receptor (NGFR) plays an essential role in the survival and maintenance of sympathetic and sensory neurons (1, 2). Nerve growth factor exerts pleiotropic effects on specific target cells during the development of the peripheral nervous system (3, 4) and these pleiotropic effects depend upon interaction of nerve growth factor with NGFR; NGFR has been detected on sensory and sympathetic neurons (5–7), human melanoma (8) and neuroblastoma (9) cells, and a rat adrenal tumor cell line (10, 11); *in vivo* it is expressed on cells derived from the neural crest (1, 2).

Using monoclonal antibodies directed against NGFR (12) to identify mouse L cells expressing NGFR after gene transfer, genomic clones for NGFR have been isolated (37). Complementary DNA (cDNA) clones were isolated from a A875 cDNA library by using genomic fragments as probes, and maps of the genomic locus and cDNA clones have been generated (37).

Growth factor receptors are of particular interest due to their central position in signal transduction pathways; and NGFR is of special interest because of its limited distribution in developing tissue, its expression (or overexpression) in melanoma cells, and the range of pleiotropic effects of the nerve growth factor–receptor interaction.

It was of particular interest to determine the location of this receptor locus since the v-*erbB* oncogene appears to have been derived from a portion of the epidermal growth factor receptor gene (13), and the oncogene carried by the McDonough strain of feline sarcoma virus has been reported to be related to the receptor for the macrophage growth factor CSF-1 (14). Additionally, knowing the position of a gene in the human genome may provide clues to possible association of that gene with particular malignancies, other genetic lesions, or predispositions. By a combination of somatic cell hybrid analysis, *in situ* hybridization, and regional mapping in somatic cell hybrids containing translocated chromosomes, the NGFR locus has been shown to reside close to, in cytogenetic terms, and distal to the chromosome 17 breakpoint seen in the t(15;17)(q22;q21) associated with acute promyelocytic leukemia (APL) (15, 16).

MATERIALS AND METHODS

Molecular Probes. Genomic clones of the human NGFR locus have been isolated through gene transfer and subsequent rescue of the transforming DNA (37). A partial restriction map of the genomic locus has been generated (37) and is depicted in Fig. 1. The 5-kilobase (kb) *Bam*HI fragment adjacent to the *Alu* sequence (Fig. 1, probe A) was used to screen a λgt10 cDNA library made from cellular RNA isolated from A875, a human melanoma cell line expressing high levels of NGFR (8), and NGFR cDNA clones were isolated (37). Two cDNA clones pSPIF (Fig. 1, probe B) and p104 (Fig. 1, probe C) were used in this study. Probes A and B detect the same regions of the NGFR locus after *Hin*dIII (or *Eco*RI) digestion. Probe B was used for somatic cell hybrid analysis and for *in situ* hybridization studies, and probes B and C were used to study the organization of the NGFR locus in DNA samples derived from leukemias. Other probes used in these studies were as follows: a human c-erbA (cHerbA) cDNA (18); a human homeobox homolog, C1 (19), which maps to human chromosome 17 (K.H., J. Hurvitz, E. Boncinelli, C.M.C., unpublished data); and a human p53 cDNA Hp53, which contains the entire coding region for the p53 gene inserted into pBR322 (20). In most cases, entire plasmids were used as probes; probe A was cleaved by *Bam*HI from the λR9 genomic DNA, electrophoresed in agarose gel, purified from agarose (21). All probes for Southern blots were labeled by nick-translation (22) by using one or several [α-^{32}P]deoxynucleotide triphosphates.

Cells. Isolation, propagation, and characterization of parental cells and somatic cell hybrids used in this study have been described (18, 23–27). All hybrids were characterized for expression of enzyme markers assigned to each of the human chromosomes (23). Some hybrid clones were karyotyped by trypsin/Giemsa and/or G-11 banding methods

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Abbreviations: NGFR, nerve growth factor receptor; APL, acute promyelocytic leukemia; kb, kilobase(s); bp, base pair(s).

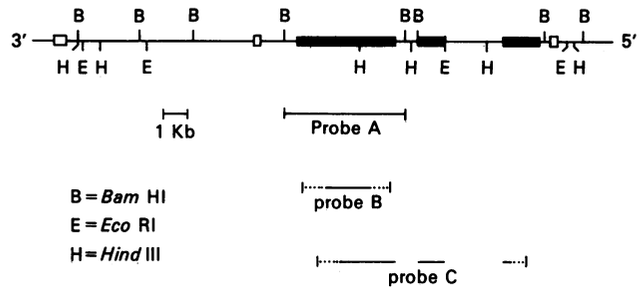

FIG. 1. Provisional restriction map of NGFR locus to show derivation of genomic and cDNA probes. Probe A was a 5-kb *Bam*HI–*Bam*HI restriction enzyme fragment derived from the genomic clone λR9; probe B was a 2.2-kb cDNA inserted into SP64 at the *Eco*RI site; probe C was a 3.1-kb cDNA inserted into SP64 at the *Eco*RI site. Dotted lines indicate that the exact endpoints of probes B and C have not yet been mapped. Open boxes are regions of the locus containing *Alu* repeats; closed boxes represent coding regions of the locus.

as described (23). In addition, the presence of specific human chromosomes in many of the mouse–human hybrids have been confirmed by DNA hybridization by using probes for genes assigned to specific human chromosomes (18, 23–27).

Tumor cells from two patients with APL (designated C and S) with the characteristic t(15;17)(q22;q21) marker chromosomes (15, 16) and one patient with undifferentiated acute leukemia, designated L, with a t(17;21)(q21;q22) chromosome (18, 28) were also studied. The 275S hybrid cell line has been described and was isolated after fusion of the human leukemia L tumor cells with mouse L-M(TK$^-$) cells. The only human chromosome retained in this hybrid is a 21q$^+$ (21pter–21q22::17q21–17qter) chromosome on a mouse cell background (18).

Southern Blot Analysis. DNAs from human peripheral blood lymphocytes, human leukemia cells, mouse cell lines, and mouse–human hybrid cell lines were extracted by cell lysis, proteinase K digestion, phenol extraction, and ethanol precipitation. Cellular DNAs were digested with an excess of appropriate restriction enzymes, sized in 0.8% agarose gels and transferred to nitrocellulose or nylon filters as described by Southern (29). Hybridization was carried out in 50% (vol/vol) formamide, 4× NaCl/Cit (1× NaCl/Cit is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), 0.2 mg of sonicated salmon sperm DNA per ml, 1× Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone) at 42°C for 15 hr. Some hybridizations were performed at 68°C without formamide. After hybridization, filters were washed and exposed to Kodak XAR-5 film with intensifying screens.

***In Situ* Hybridization.** Metaphase spreads were prepared from normal human male lymphocyte cultures stimulated with phytohemagglutinin for 72 hr. NGFR probe B cDNA fragment was nick-translated with [^{3}H]dCTP (62 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear), [^{3}H]dGTP (39.9 Ci/mmol), [^{3}H]dTTP (100.1 Ci/mmol), and [^{3}H]dATP (51.9 Ci/mmol). The techniques used for *in situ* hybridization were essentially as described by Harper and Saunders (30). Chromosome preparations were treated with pancreatic RNase A (Sigma) and then denatured in 70% (vol/vol) formamide in 2× NaCl/Cit (pH 7.0) at 70°C for 2 min. The chromosome preparations were then hybridized with ^{3}H-labeled probe B NGFR DNA (specific activity 3×10^7 cpm/μg) at a concentration of 140 to 280 ng/ml in 50% (vol/vol) formamide/2× NaCl/Cit/20 mM NaH_2PO_4/10% (wt/vol) dextran sulfate (Pharmacia), pH 7.0, for 20 hr at 37°C. A 300-fold excess of sonicated salmon sperm DNA was included as carrier. Slides were thoroughly rinsed in 50% (vol/vol) formamide, 2× NaCl/Cit at 39°C, exposed to Kodak NTB2 nuclear track emulsion for 16 days at 4°C, and developed with Kodak Dektol at 15°C. The chromosomes were then G banded essentially as described by Cannizzaro and Emanuel (31) with a mixture of 6 parts of borate buffer (50 mM Na_2SO_4/2.5 mM $Na_2B_4O_7$, pH 9.2) to 1 part of Wright's/Giemsa stain solution (2.4 g of Wright's stain per liter/1.4 g of Giemsa stain per liter in methanol).

RESULTS

Localization of NGFR Gene to Human Chromosome 17 by Somatic Cell Hybrid Analysis. In initial experiments the NGFR genomic fragment (Fig. 1, probe A) was hybridized to Southern blots of *Hin*dIII cleaved parental and hybrid cell DNAs that retain specific subsets of human chromosomes. Although this fragment lacked *Alu* repeats (37), it displayed nonspecific background hybridization to mouse and hybrid DNAs in addition to the specific NGFR fragment (not shown); therefore, the probe B cDNA plasmid, which detects the same portion of the NGFR locus as does probe A (see Fig. 1) was employed for hybrid panel screening. An example of a Southern blot, in which 16 hybrid cell DNAs are represented, is presented in Fig. 2; human control DNA (Fig. 2, lane 2) and DNA from 10 hybrids (Fig. 2, lanes 3–9 and 16–18) are positive for the 9.0-kilobase pair (kbp) human-specific NGFR fragment generated by *Hin*dIII cleavage; DNA from 3 other hybrids (Fig. 2, lanes 11, 13, and 14) is faintly positive for the fragment and on long exposures of the same filter are clearly positive (not shown). The mouse NGFR homolog is not detected under the hybridization conditions used. These hybrids and another six hybrids (not shown) were also tested for the presence of the NGFR gene after *Eco*RI cleavage and *Hin*dIII cleavage (for the additional six). Since human chromosome 17 is the only human chromosome present in the hybrid represented in Fig. 2, lane 9, and all other positive hybrids retained human chromosome 17 (or 17q; see Fig. 2, lane 7), while negative hybrids did not, the human NGFR locus is on human 17 (probably on the long arm). The somatic cell hybridization data is summarized in Table 1 and clearly demonstrates a correlation between presence of the NGFR gene and human chromosome 17 in 24 mouse–human hybrids; all other chromosomes show numerous discordancies. Presence of human chromosome 17 in the hybrid cells was confirmed by using DNA probes for three other genes that

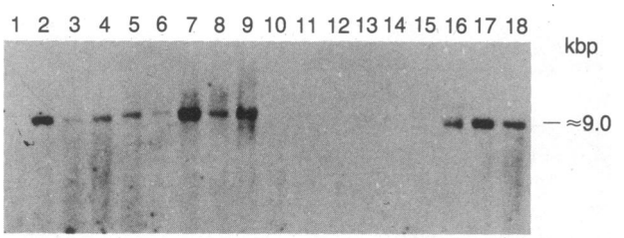

FIG. 2. NGFR locus is on human chromosome 17. Somatic cell hybrid DNA panel was probed with NGFR cDNA clone, probe B. DNA was digested with restriction enzyme *Hin*dIII, fractionated in agarose, transferred to nylon filter, and hybridized to nick-translated ^{32}P-labeled probe B DNA. DNA (10 μg/lane) from murine cell line (lane 1); normal human PBLs (lane 2). DNA (10 μg/lane) from hybrids (lanes 3–18). Hybrids retained the following human chromosomes: 2, 5, 12, 16, 17, 20 (lane 3); 2, 5, 17 (lane 4); 1, 3, 4, 5, 6, 7, 8, 9, 10, 13, 14, 17, 18, 20, 22, X (lane 5); 1, 3, 4, 5, 7, 8, 9, 10, 13, 14, 17, 20, 22, X (lane 6); 6, 7, 17q, 21 (lane 7); 1, 3, 4, 5, 6, 10, 11, 14, 17, X (lane 8); 17 (lane 9); 7 (lane 10); 5, 7, 8, 9, 12, 13, 14, 15, 17 (in a few cells), 21, 22 (lane 11); 7p11.4→qter (lane 12); 3, 4, 5, 6, 11, 13, 14, 15, 17 (in a few cells), 18, X (lane 13); 5, 6, 13, 14, 17 (in a few cells), 18, 20, 22, X (lane 14); 1, 2, 3, 4, 7, 9, 10, 11, 12, 14, 15, 16, 18, 19, 20, 21, X (lane 15); 1, 3, 4, 5, 6, 10, 11, 14, 17, X (lane 16); 9, 12, 13, 14, 17, 21, 22 (lane 17); 5, 8, 14, 15, 17, 18, 19, 21, 22, X (lane 18).

Table 1. Correlation of presence of NGFR gene and specific human chromosomes in 24 mouse–human hybrids

Human chromosome	Hybrid clones, no. NGFR gene/chromosome retention +/+	−/−	+/−	−/+	Number discordant
1	6	4	13	1	14
2	2	4	17	1	18
3	8	3	11	2	13
4	10	3	9	2	11
5	13	4	6	1	7
6	8	5	11	0	11
7	8	2	11	3	14
8	7	5	12	0	12
9	8	4	11	1	12
10	7	4	12	1	13
11	3	4	16	1	17
12	4	4	15	1	16
13	12	5	7	0	7
14	13	2	6	3	9
15	4	3	15	2	17
16	3	4	16	1	17
17	19	5	0	0	0
18	7	4	12	1	13
19	1	4	18	1	19
20	9	3	10	2	12
21	4	4	15	1	16
22	10	4	9	1	10
X	10	3	9	2	11

DNA, from a panel of hybrid cells characterized for the presence of specific human chromosomes by isozyme analysis and, in some cases, by karyotypic analysis and by DNA·DNA hybridization that used DNA probes for genes assigned to specific chromosomes, was analyzed for the presence of the human NGFR gene as shown in Fig. 2.

have been mapped to human chromosome 17 (18–20, 32–35), c-*erbA* (18), a human homeobox homolog (19), and a human p53 cDNA (20).

Localization of NGFR Gene to 17q12–17q22 by *in Situ* Hybridization. The assignment of the NGFR locus to human chromosome 17 has been confirmed and refined by *in situ* hybridization of ^{3}H-labeled NGFR plasmid DNA (probe B) to metaphase chromosomes from peripheral blood lymphocytes of a normal male. After autoradiography, metaphase spreads were analyzed for grain localization. An example of one such spread is shown in Fig. 3 (*Upper*). About 25% of all grains were located on the long arm of chromosome 17. Over 84% of the 17q grains were between 17q12 and 17q22, with most grains at 17q21. A histogram depicting the silver grain distribution along the human chromosomes is shown in Fig. 3 (*Lower*). The long arm of chromosome 17 represents approximately 2.2% of the haploid genome, and our observation that more than 20% of the human NGFR probe hybridization was localized to the proximal half of this chromosome segment is highly significant ($P < 0.001$). Thus, cytological hybridization localizes the human NGFR gene to the region between 17q12 and 17q22, with most grains at 17q21.

Localization of the NGFR Locus to the Distal Side of the Leukemia-Associated Chromosome 17 Breakpoint. The localization of the human NGFR locus at 17q12–17q22 places it close to the region containing one of the breakpoints in the t(15;17)(q22;q21) associated with APL. The c-*erbA* locus has been mapped to chromosome 17 proximal to the 17q21 APL breakpoint (18, 32), but the gene locus which is at the chromosome 17 APL-associated breakpoint, which may be interrupted by translocation, has not yet been identified.

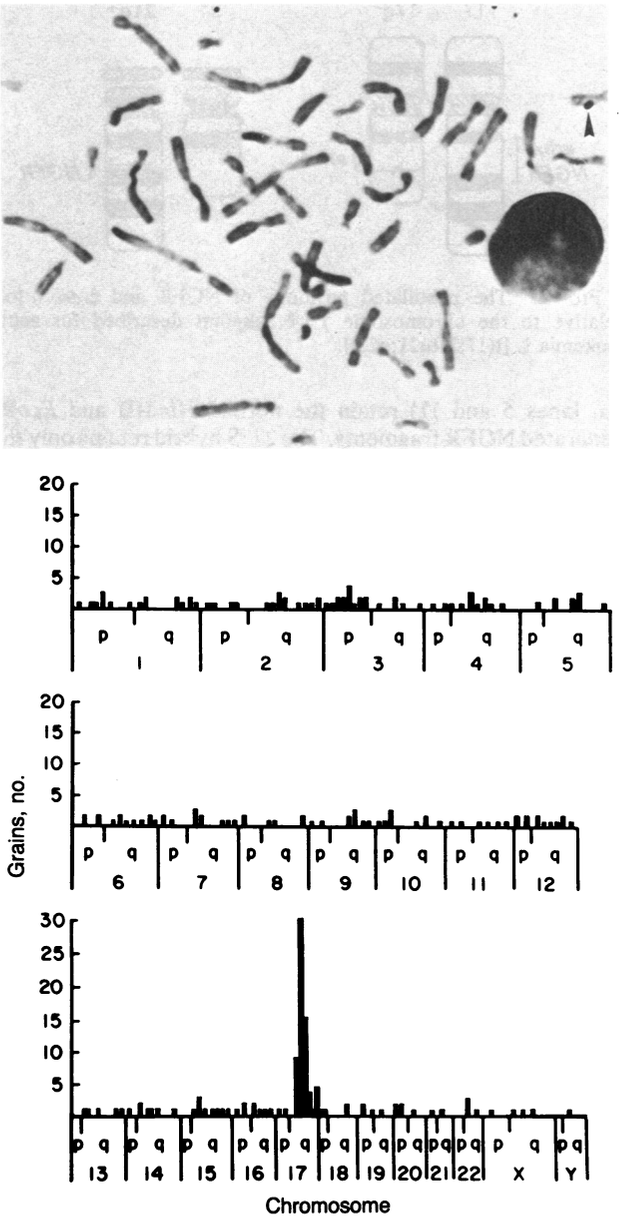

FIG. 3. Localization of NGFR gene in the human genome by *in situ* hybridization analysis. (*Upper*) Photograph of a G-banded lymphocyte metaphase spread hybridized with the human NGFR probe B. An arrow indicates a grain found over chromosome 17. (*Lower*) Diagram showing the grain distribution in 110 metaphases. The abscissa represents the chromosomes in their relative size proportion; the ordinate shows the number of silver grains. The distribution of 262 grains on 110 spreads was scored; 55 were found over 17q12–17q22.

Thus, it was of interest to determine where the NGFR locus resides relative to the APL 17q21 breakpoint. We have carried out Southern analysis of *Hin*dIII and *Eco*RI cleaved DNAs derived from leukemia patients and from a mouse–human hybrid (275S) retaining only a 21q$^+$ (21pter–21q22::17q21–qter) chromosome (see Fig. 4) derived from a patient with undifferentiated acute leukemia (28) in a mouse L-M(TK$^-$) background (18). The breakpoint in chromosome 17 in this tumor was considered identical with that of the characteristic t(15;17) chromosome translocation breakpoint of APL (28). The leukemia-derived and hybrid DNA containing filters were hybridized with an NGFR cDNA clone (Fig. 1, probe C). Results are shown in Fig. 5*a*. The leukemia DNAs (Fig. 5*a*, lanes 3, 4, and 8–10) and hybrid 275S (Fig.

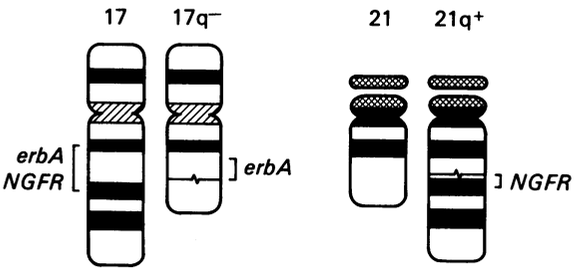

FIG. 4. The postulated positions of NGFR and c-*erbA* loci relative to the chromosome 17 breakpoint described for acute leukemia L [t(17;21(q21;q22)].

5*a*, lanes 5 and 11) retain the normal *Hin*dIII and *Eco*RI generated NGFR fragments. The 275S hybrid retains only the distal portion of chromosome 17, as has been shown (18) by absence of the human c-*erbA* gene in this hybrid. The longer NGFR cDNA clone, probe C, detects four *Hin*dIII fragments in the NGFR locus (see Fig. 5*a*). Three of these bands (3.6, 3.0, and 1.8 of which 3.6 is the most 5′-proximal band with the

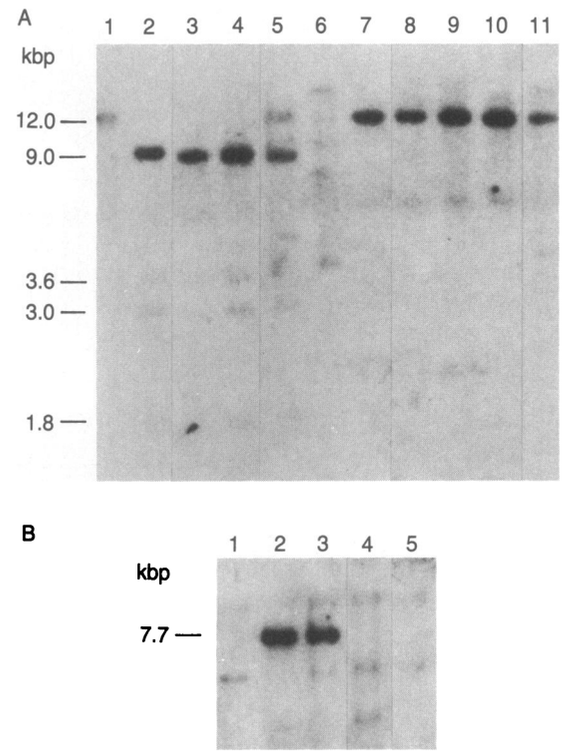

FIG. 5. NGFR locus in acute leukemias. Leukemia DNAs were cleaved with restriction enzymes and probed with cDNA probe C. (*a*) DNA (10 μg/lane) from the following cells: mouse cell line (lane 1); human PBL (lane 2); APL C (lane 3); acute leukemia L (lane 4); 275S hybrid cell (lane 5); mouse cell line (lane 6); human PBL (lane 7); APL C (lane 8); acute leukemia L (lane 9); APL S (lane 10); 275S hybrid cell (lane 11). DNA in lanes 1–5 was digested with *Hin*dIII, and DNA in lanes 6–11 was digested with *Eco*RI. Note that acute leukemia L was the human parental cell for the 275S hybrid. Digested DNAs were electrophoresed, transferred to nylon filters, and hybridized to ^{32}P-labeled NGFR probe C DNA. (*b*) DNA (10 μg/lane) from the following cells: mouse cell line (lane 1); human peripheral blood lymphocytes (lane 2); hybrid retaining only human chromosome 17 (lane 3); hybrid retaining human chromosome 6, 7, 17q, 21 (lane 4); hybrid 275S was cleaved with restriction enzyme *Bam*HI (lane 5). DNA was separated electrophoretically, transferred to nylon filter, and hybridized to the ^{32}P-labeled Hp53 cDNA clone. NGFR for hybrids (lanes 3 and 4) is shown in Fig. 2, lanes 9 and 7, respectively. Approximate molecular sizes of human NGFR and p53-specific fragments are shown beside the figure.

3.0- and 1.8-kbp bands falling between the 3.6 and 9.0-kbp band on the genomic locus; see Fig. 1) are detected only weakly by probe C (Fig. 5*a*). On longer exposures of the x-ray film shown in Fig. 5*a*, the 3.6-, 3.0-, and 1.8-kbp *Hin*dIII fragments are seen in all three leukemia DNAs and in the 275S DNA. Note that the 275S DNA also exhibits mouse-specific bands (compare lane 1 to lane 5 and lane 6 to lane 11). Thus, the NGFR probe C, the longest cDNA clone investigated, does not detect rearrangement in the NGFR locus with the restriction enzymes thus far tested.

As mentioned briefly above, we have routinely rehybridized NGFR blots with probes for other genes that have been mapped to chromosome 17. We find that the human p53 gene is absent from the 275S hybrid and is thus proximal to the 17q21 breakpoint of this tumor (see Fig. 5*b*, lane 5). Another hybrid, which retains the long arm of chromosome 17 but has lost the short arm, retains the NGFR gene (Fig. 2, lane 7) but is negative for the human p53 gene (Fig. 5*b*, lane 4), indicating that p53 maps to the short arm of chromosome 17 in agreement with a report (36). In fact, *in situ* hybridization localizes the human p53 gene to 17p (38). The leukemia DNAs thus far tested from two patients with APL (tumors C and S) and one patient with acute leukemia (tumor L) show a germline configuration for the NGFR region detected by probes B and C (Fig. 5*a* and unpublished data). Until probes derived from the 5′ and 3′ regions of the NGFR locus are tested, we cannot determine if the NGFR locus is directly altered by the t(15;17) translocation of APL; but our data from the t(17;21) translocation suggest that most, if not all, of the locus is distal to the breakpoint on chromosome 17.

DISCUSSION

The NGFR gene locus has been molecularly cloned by identification of transfectants that express NGFR (37) and characterized by restriction enzyme mapping of genomic and cDNA clones. This gene has now been localized in the human genome to chromosome region 17q12–q22 by correlation between presence of the gene and presence of specific human chromosomes in a panel of mouse–human hybrids and by *in situ* hybridization of the labeled cDNA clone to metaphase chromosomes of a normal subject. The gene has been further localized as distal, and probably closely distal in cytogenetic distances, to the chromosome 17q21–q22 breakpoint observed in the t(15;17) chromosome associated with APL.

To determine which regions of chromosome 17 were present in each of the hybrid clones used for these studies, we have also determined the segregation pattern of three other genes that have been assigned to chromosome 17. We have confirmed that the human *erbA* locus is on 17 proximal to the APL chromosome 17 breakpoint; our data also indicate that a new member of the human homeobox gene cluster (19) is distal to the same breakpoint and that the human p53 locus is on the short arm of chromosome 17.

In preliminary investigations of two cases of APL with the typical t(15;17) translocation, we have also determined that the internal NGFR region detected by the available NGFR cloned probes is intact and that the same is true in the t(17;21) translocation observed in one case of poorly differentiated acute leukemia. It is possible that rearrangements indicating an involvement of the NGFR locus in the 17q21–q22 breakpoint will be detected by using cloned fragments from the 5′ or 3′ region of the NGFR locus. It will also be of interest to determine the genomic organization (germline, rearranged, amplified) of the NGFR locus in tumors of neural crest origin.

By virtue of its genomic location, the NGFR gene joins a linkage group whose other members, gene for thymidine kinase, galactokinase, homeobox genes, are linked in mouse (on chromosome 11) and man (17). Thus the murine NGFR gene may be located on mouse chromosome 11. Due to its

linkage to the thymidine kinase gene, the NGFR gene is likely to be retained in somatic cell hybrids in which the thymidine kinase gene has been selectively retained, thus facilitating studies of expression of this gene in various hybrid cells. Whether the NGFR gene is very closely linked to the thymidine kinase gene could be determined in transfection experiments in which the human thymidine kinase gene is selectively retained in a mouse cell background.

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1. Levi-Montalcini, R. & Angeletti, P. U. (1963) *Dev. Biol.* **7,** 655–659.
2. Levi-Montalcini, R. & Angeletti, P. U. (1968) *Physiol. Rev.* **48,** 534–569.
3. Thoenen, H. & Barde, Y.-A. (1980) *Physiol. Rev.* **60,** 1284–1335.
4. Yankner, B. A. & Shooter, E. M. (1982) *Annu. Rev. Biochem.* **51,** 845–868.
5. Banerjee, S. P., Snyder, S. H., Cuatrecasas, P. & Greene, L. A. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2519–2523.
6. Herrup, K. & Shooter, E. M. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3884–3888.
7. Frazier, W. A., Boyd, L. F. & Bradshaw, R. A. (1974) *J. Biol. Chem.* **249,** 5513–5519.
8. Fabricant, R. F., DeLarco, J. E. & Todaro, G. J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 565–569.
9. Sonnenfeld, K. H. & Ishii, D. N. (1982) *J. Neurosci. Res.* **8,** 375–391.
10. Landreth, G. E. & Shooter, E. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4751–4755.
11. Herrup, K. & Thoenen, H. (1979) *Exp. Cell Res.* **121,** 71–78.
12. Ross, A. H., Grob, P., Bothwell, M., Elder, D. E., Ernst, C. S., Marano, W., Ghrist, B. F. D., Slemp, C. C., Herlyn, M., Atkinson, B. & Koprowski, H. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6681–6685.
13. Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlesinger, J. & Waterfield, M. D. (1981) *Nature (London)* **307,** 521–527.
14. Sherr, C. J., Rettenmeler, C. W., Sacca, R., Roussel, M. F., Look, A. T. & Stanley, E. R. (1985) *Cell* **41,** 665–676.
15. Rowley, J. D., Golomb, H. M., Vardiman, J., Fukukara, S., Dougherty, C. & Potter, D. (1977) *Int. J. Cancer* **20,** 869–872.
16. Larson, R. G. (1984) *Am. J. Med.* **76,** 827–841.
17. O'Brien, S. J., ed. (1984) *Genetic Maps* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol. 3.
18. Dayton, A. I., Selden, J. R., Laws, G., Dorney, D. J., Finan, J., Tripputi, P., Emanuel, B. S., Rovera, G., Nowell, P. C. & Croce, C. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4495–4499.
19. Simeone, A. & Boncinelli, E. (1986) *Cell*, in press.
20. Zakut-Houri, R., Bienz-Tadmor, B., Givol, D. & Oren, M. (1985) *EMBO J.* **4,** 1251–1255.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
22. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
23. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C. & Croce, C. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7824–7827.
24. Cooper, C. S., Park, M., Blair, D. G., Tainsky, M. A., Huebner, K., Croce, C. M. & Vande Woude, G. F. (1984) *Nature (London)* **311,** 29–33.
25. Huebner, K., Palumbo, A. P., Isobe, M., Kozak, C. A., Monaco, S., Rovera, G., Croce, C. M. & Curtis, P. J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3790–3793.
26. Croce, C. M., Isobe, M., Palumbo, A., Puck, J., Ming, J., Tweardy, D., Erikson, J., Davis, M. & Rovera, G. (1985) *Science* **227,** 1044–1047.
27. Isobe, M., Huebner, K., Erikson, J., Peterson, R. C., Bollum, F. J., Chang, L. M. S. & Croce, C. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5836–5840.
28. Nowell, P. C., Wilmoth, D., Hoxie, J. & Lyss, A. P. (1984) *Cancer Genet. Cytogenet.* **12,** 51–55.
29. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
30. Harper, M. E. & Saunders, G. F. (1981) *Chromosoma* **83,** 431–439.
31. Cannizzaro, L. A. & Emanuel, B. (1984) *Cytogenet. Cell Genet.* **38,** 308–309.
32. Spurr, N. K., Solomon, E., Johnson, M., Sheer, D., Goodfellow, P. N., Bodmer, W. F. & Vennstrom, B. (1984) *EMBO J* **3,** 159–163.
33. Joyner, A. L., Lebo, R. V., Kan, Y. W., Tijan, R., Cox, D. R. & Martin, G. R. (1985) *Nature (London)* **314,** 173–175.
34. Rabin, M., Hart, C. P., Ferguson-Smith, A., McGinnis, W., Levine, M. & Ruddle, F. H. (1985) *Nature (London)* **314,** 175–177.
35. Le Beau, M. M., Westbrook, C. A., Diaz, M. O., Rowley, J. D. & Oren, M. (1985) *Nature (London)* **316,** 826–828.
36. Benchimol, S., Lamb, P., Crawford, L. V., Sheer, D., Shows, T. B., Bruns, G. A. P. & Peacock, J. (1985) *Somatic Cell Mol. Genet.* **11,** 505–510.
37. Chao, M., Bothwell, M., Ross, A. R., Koprowski, H., Lanahan, A., Buck, C. R. & Sehgal, A. (1986) *Science*, in press.
38. Isobe, M., Emanuel, B. S., Givol, D., Oren, M. & Croce, C. M. (1986) *Nature (London)*, in press.