induced rat neuroblastoma has been detected by DNA transfection analysis (*11*). This oncogene, designated *neu*, appears to encode a protein immunologically related to the EGF receptor (*12*). Whether the MAC117 coding sequence and *neu* represent the same or different cellular genes awaits further characterization.

Overexpression of proto-oncogenes can cause cell transformation in culture and may function in the development of human tumors. Amplification of a normal *ras* gene or its increased expression under the control of a retroviral long terminal repeat (LTR) induces transformation of NIH 3T3 cells (*13*). Expression of the normal human *sis*/PDGF-2 coding sequence in NIH 3T3 cells, which do not normally express their endogenous *sis* proto-oncogene, also leads to transformation (*14*). In Burkitt lymphoma, a chromosomal translocation involving *myc* places its normal coding sequence under the control of an immunoglobulin gene regulatory sequence (*15*). The resulting alteration in *myc* expression is likely to be causally related to tumor development (*16*). The observation of amplification of *myc* or N-*myc* in more malignant phenotypes of certain tumors has supported the idea that overexpression of these genes can contribute to the progression of such tumors (*8, 17*). The *erb*B/EGF receptor gene is amplified or overexpressed in certain tumors or tumor cell lines (*6*). The five- to tenfold amplification of our v-*erb*B–related gene in a mammary carcinoma suggests that increased expression of this gene may have provided a selective advantage to this tumor. The isolation of a new member of the tyrosine kinase gene family amplified in a human mammary carcinoma provides an opportunity to investigate the potential role of this gene in human malignancy.

Note added in proof: Recently, Semba *et al.* (*28*) independently detected a v-*erb*B–related gene that was amplified in a human salivary gland adenocarcinoma. Nucleotide sequence analysis of this gene indicates its identity to the MAC117 gene in the regions compared.

References and Notes

1. J. M. Bishop, *Annu. Rev. Biochem.* **52**, 301 (1983); P. H. Duesberg, *Nature (London)* **304**, 219 (1983).
2. R. F. Doolittle *et al.*, *Science* **221**, 275 (1983); M. D. Waterfield *et al.*, *Nature (London)* **304**, 35 (1983).
3. J. Downward *et al.*, *Nature (London)* **307**, 521 (1984).
4. A. Hampe, I. Laprevotle, F. Galibert, L. A. Fedele, C. J. Sherr, *Cell* **30**, 775 (1982); N. Kitamura, A. Kitamura, K. Toyoshima, Y. Hirayama, M. Yoshida, *Nature (London)* **297**, 205 (1982); M. Shibuya and H. Hanafusa, *Cell* **30**, 787 (1982); T. Yamamoto *et al.*, *ibid.* **35**, 71 (1983).
5. A. de Klein *et al.*, *Nature (London)* **300**, 765 (1982); S. J. Collins and M. T. Groudine, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4813 (1983).
6. T. A. Libermann *et al.*, *Nature (London)* **313**, 144 (1985).
7. A. Ullrich *et al.*, *ibid.* **309**, 418 (1984); Y. Xu *et al.*, *ibid.*, p. 809; C. R. Lin *et al.*, *Science* **224**, 843 (1984).
8. M. Schwab *Nature (London)* **305**, 245 (1983); N. E. Kohl *et al.*, *Cell* **35**, 349 (1983).
9. R. Breathnach and P. Chambon, *Annu. Rev. Biochem.* **50**, 349 (1981).
10. J. E. Smart *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6013 (1981).
11. Alan L. Schechter *et al.*, *Nature (London)* **312**, 513 (1984).
12. J. A. Drebin, D. F. Stern, V. C. Link, R. A. Weinberg, M. I. Greene, *ibid.*, p. 545.
13. E. H. Chang, M. E. Furth, E. M. Scolnick, D. R. Lowy, *ibid.* **297**, 479 (1982).
14. A. Gazit *et al.*, *Cell* **39**, 89 (1984); M. F. Clarke *et al.*, *Nature (London)* **308**, 464 (1984).
15. R. Taub *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7837 (1982).
16. K. Nishikura *et al.*, *Science* **224**, 399 (1984).
17. M. Schwab *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4940 (1984).
18. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
19. G. M. Wahl, M. Stern, G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3683 (1979).
20. P. W. J. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977).
21. A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
22. The following abbreviations were used for amino acids: A, alanine; C, cysteine, D, aspartic acid; E, glutamic acid; F. phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.
23. H. D. Lehrach, D. Diamond, J. M. Wozney, H. Boedtker, *Biochemistry* **16**, 4743 (1977).
24. A. P. Czernilofsky *et al.*, *Nature (London)* **287**, 193 (1980).
25. E. P. Reddy, M. J. Smith, A. Srinivasan, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3623 (1983).
26. A. Ullrich *et al.*, *Nature (London)* **313**, 756 (1985).
27. D. J. Lipman and W. R. Pearson, *Science* **227**, 1435 (1985).
28. K. Semba, N. Kamata, K. Toyshima, T. Yamamoto, *Proc. Natl. Acad. Sci. U.S.A.*, in press.

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The *neu* Gene: An *erb*B-Homologous Gene Distinct from and Unlinked to the Gene Encoding the EGF Receptor

Abstract. *The* neu *oncogene, identified in ethylnitrosourea-induced rat neuroglioblastomas, had strong homology with the* erb*B gene that encodes the epidermal growth factor receptor. This homology was limited to the region of* erb*B encoding the tyrosine kinase domain. It was concluded that the* neu *gene is a distinct novel gene, as it is not coamplified with sequences encoding the EGF receptor in the genome of the A431 tumor line and it maps to human chromosome 17.*

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Rat neuroglioblastomas induced by exposure in utero to ethylnitrosourea frequently carry an oncogene detectable upon transfection into NIH 3T3 mouse cells (*1*). This oncogene (which we have termed *neu*) was found to be related to c-*erb*B (*2*), a gene that encodes the receptor for epidermal growth factor (EGF-r) (*3*). The *neu* oncogene induces the synthesis of a tumor antigen, p185, which is serologically related to the EGF-r (*2*).

Southern blot analysis of rat DNA after Eco RI digestion revealed at least two *erb*B-homologous segments, one of which contained the *neu* oncogene in a biologically active form (*2*). It remained unclear whether the same or other DNA segments encode the EGF-r. Other analysis uncovered differences between the products of the two genes. While polyclonal sera to the EGF-r recognized p185, monoclonal antibodies to p185 did not react with the EGF-r. Moreover, there was an apparent molecular weight difference of 15,000 daltons between the two proteins (*2*).

These data raised several possibilities regarding the relationship between the *neu* and c-*erb*B genes. The *neu* oncogene might be a mutated allele of the normal c-*erb*B gene, or it might be derived from a normal gene, the sequences of which overlap with those of c-*erb*B. Alternatively, the *neu* oncogene might have arisen from a gene that is totally separate and distinct from *erb*B.

We used three subclones of human c-*erb*B complementary DNA (cDNA) (*4*) and a 0.7-kilobase (kb) subclone of the v-*erb*B oncogene that had been transduced by the genome of avian erythroblastosis virus (*5*) for these studies. All of the *neu* oncogene lies within a 34-kb Eco RI segment that is present in the genomes of normal and tumor rat cells as well as in mouse NIH 3T3 cells that have acquired a *neu* oncogene via transfection (*2*). We

tested whether the *erb*B subclones encoding various portions of the EGF-r were closely related to sequences that are present within this large Eco RI segment.

Figure 1A shows a Southern blot analysis in which an *erb*B subclone (64-3), which encodes the amino acids comprising the protein kinase domain of the human EGF-r (*4*, *6*, *7*), was used as a probe. This probe hybridized to several DNA segments in addition to the 34-kb segment in the genomes of normal rat cells, of B104 rat neuroglioblastoma cells, and of mouse NIH 3T3 cells. The DNA of a transfected NIH 3T3 line, B1041-2, which carries the *neu* oncogene from a rat neuroglioblastoma, had a novel *erb*B-homologous segment of more than 40 kb. This 40-kb Eco RI segment arose from the 34-kb segment after two rounds of transfection (*2*). Its larger size reflects the loss of Eco RI sites normally flanking *neu*, which occurred during the transfection process. We continued to use B1041-2 since its Eco RI segment (of rat origin) was easily distinguishable from the mouse homolog that is endogenous to these cells.

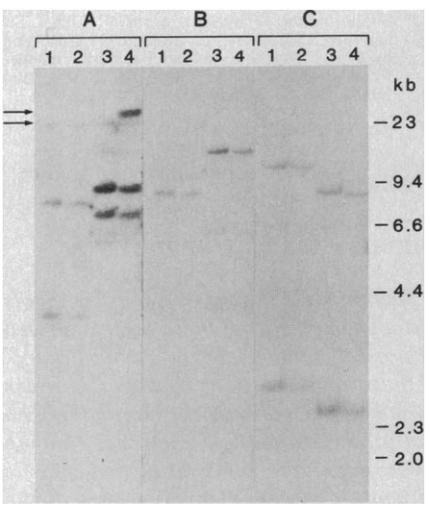

Fig. 1. Hybridization of *erb*B subclones to Eco RI digests of genomic DNA from the following sources: (lane 1) normal rat liver; (lane 2) B104 rat neuroglioblastoma cells; (lane 3) NIH 3T3 cells; (lane 4) B104-1-2 cells, secondary transfectants of B104. High molecular weight DNA (10 μg) was digested with Eco RI, fractionated by electrophoresis in 1 percent agarose gels, and transferred to nylon filters (Zeta Bind). The filters were incubated at low stringency [30 percent formamide, 5× standard saline citrate phosphate EDTA (SSCPE), 42°C] for 36 hours with one of the following ^{32}P-dCTP nick-translated cloned DNA preparations: (A) 64-3, (B) 64-1, (C) 62-1. The filters were then washed three times at 20°C for 10 minutes and for 4 hours at 50°C in 2× SSCPE and exposed to Kodak X-Omat AR film for 3 days at −70°C. The *neu*-containing Eco RI segments are shown by arrows (left); Hind III–digested λ DNA was used for the size markers (right).

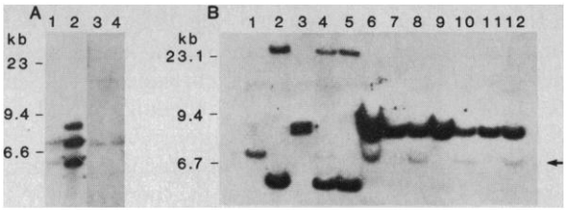

Fig. 2. (A) Southern blot analysis of human sequences homologous to *neu*. DNA was isolated from HeLa cells (lanes 1 and 3) and A431 cells (lanes 2 and 4) and treated as in Fig. 1. The filters were hybridized with either ^{32}P-dCTP nick-translated 64-1 (lanes 1 and 2) or *neu*-specific Bam HI probe (lanes 3 and 4). Conditions of hybridization and washes were as described in Fig. 1. (B) Southern analysis of sequences homologous to *neu* in human × rodent somatic cell hybrids. Somatic cell hybrids of five different series were used (*16*). Ten micrograms of DNA from hybrid, mouse, and human cells and 2 μg of hamster DNA were digested with Eco RI, separated in 0.7 percent agarose gels, and transferred to nitrocellulose filters. The filters were hybridized with the 4-kb Bam HI *neu* probe. (Lane 1) Human lymphoblastoid cell line; (lane 2) mouse NIH 3T3 cells; (lane 3) Chinese hamster cell line; (lane 4) subclone of a mouse × human hybrid in which a rearranged chromosome 17 contained only region q21-qter (retained by growth in media containing hypoxanthine, aminopterin, and thymidine); (lane 5) same subclone as in lane 4, but missing this rearranged chromosome (after counter-selection in bromodeoxyuridine-containing media); (lanes 6, 8, 10, and 12) Chinese hamster clones containing chromosome 17; (lanes 5, 7, 9, and 11) cell clones lacking human chromosome 17. The arrow at the right shows the Eco RI segment of human origin.

To further explore the structural relations of the *neu* and EGF-r genes, a second filter was hybridized with a 1.8-kb cDNA subclone 64-1. This subclone encodes the amino acids comprising the putative binding and transmembrane domains of the EGF-r (*4*, *8*). While this probe recognized homologous segments in the rat and mouse genomes, it did not react with the Eco RI segment carrying *neu* (Fig. 1B). This failure to anneal is reflected not only in the genomes of rat neuroglioblastoma cells and the derived transfectant but in the genomes of normal rat fibroblasts as well. Thus, the segment of DNA containing all of the *neu* gene in both its normal and oncogenic forms was not homologous to the 5′ coding sequences of the EGF-r.

The differences between the two genes were also defined by Southern blot analyses in which another *erb*B cDNA subclone, 62-1, was used. This subclone contains 600 nucleotides of 3′ untranslated sequences as well as sequences encoding the 170 amino acids of the intracellular COOH-terminus of the EGF-r (*4*, *6*). This region of the EGF-r contains the major sites for autophosphorylation in vivo and in vitro (*9*). This probe hybridized with DNA segments present within the rat and mouse genomes, but not with the Eco RI segments known to contain *neu* (Fig. 1C).

We further delimited the region within the avian viral gene v-*erb*B, which is homologous in the two genes, by means of a probe that includes sequences encoding a small region of 50 amino acids upstream (NH_2 terminal) to the start of the protein kinase domain (*6*). Even this probe did not hybridize to the large Eco RI DNA segment carrying *neu*. These results indicate that the region of strong homology of the *erb*B and *neu* genes is limited to sequences encoding the protein kinase domain of the EGF-r.

While providing strong evidence that the *erb*B and *neu* genes encode essentially distinct proteins, this work did not resolve whether the two genes were separate from one another, or overlapped and shared certain exons. Therefore, we

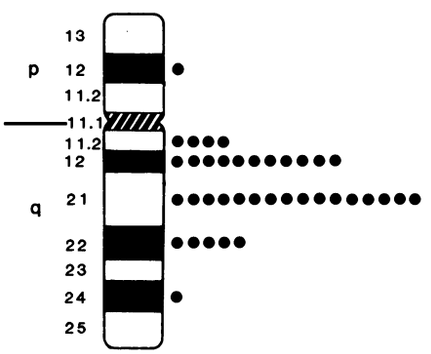

Fig. 3. Autoradiographic silver grain–distribution along chromosome 17 representing specific hybridization to *neu* (*17*). Human metaphase and prometaphase chromosomes were prepared from methotrexate-synchronized peripheral lymphocyte cultures (*18*) of two normal individuals. The 4.0-kb Bam HI segment of *neu* was nick-translated with [^{3}H]dCTP, [^{3}H]dATP, and [^{3}H]dTTP to a specific activity of 2 × 10^7 count/min per microgram of DNA; in situ hybridization was carried out as described (*19*). The probe was hybridized to chromosome preparations on glass slides at a concentration of 25 or 50 ng/ml for 15 hours at 37°C. Emulsion-coated slides were incubated for 14 to 18 days at 4°C. Chromosomes were stained with quinacrine mustard dihydrochloride and photographed under a fluorescence microscope. The chromosomes were then G-banded with Wright's stain and a second photograph was taken of the previously selected cells. G-banded chromosomes were analyzed for silver grain distribution. Each dark circle represents a single grain.

analyzed the genome of the human A431 carcinoma cell line, which contains a highly amplified number of copies of the gene encoding the EGF-r (*4, 10, 11*). To determine whether the *neu* gene was coamplified in this cell line, a set of nitrocellulose filters was prepared containing Eco RI–digested DNA isolated from A431 cells and from HeLa cells; the DNA from HeLa cells, another human tumor line, was used as a control. When this filter was hybridized with the EGF-r cDNA subclone 64-1, the A431 cell line was observed to contain amplified DNA segments homologous to the probe (Fig. 2A). We, and other investigators (*4, 10*), have found that segments homologous to other domains of c-*erb*B are also amplified. In initial studies, we hybridized a duplicate filter with a 4.0-kb Bam HI genomic subclone of the *neu* gene and found that this segment hybridized preferentially to *neu*. The human genome contained a 7.2- and 16-kb Eco RI segment homologous to this *neu* probe; neither of these segments was amplified in the A431 cell line (Fig. 2A). Thus, *neu* has not been coamplified with c-*erb*B in A431 cells, suggesting that the two genes are separate and nonoverlapping.

We determined the chromosomal position of *neu* in order to further define its relationship to *erb*B. The human *erb*B gene encoding the EGF-r is located on chromosome 7, region 7p11-p13 (*12*). We first ascertained the chromosomal location of the human *neu* gene by in situ hybridization of the 4.0-kb Bam HI segment to metaphase chromosome preparations. In situ hybridization (Fig. 3) resulted in 217 silver grains on 110 metaphase cells, 36 (16.6 percent) of which were over a specific chromosomal region, 17q11.2-q22. No other sites were labeled above background. Southern blot analysis of DNA's prepared from ten human × rodent somatic cell hybrids confirmed the localization of *neu* to chromosome 17. The hybrids carrying human chromosome 17 all contain the 7.2-kb Eco RI segment (Fig. 2B) that is specific for the human *neu* gene (the 16-kb human segment being visible after longer exposure). The intense 8.0-kb band seen in Fig. 2B (lanes 3 and 6 to 12) is the hamster homolog of *neu*, which reacts strongly with the probe because of its rodent origin.

Chromosome 17 is the only chromosome with perfect concordant segregation, and other chromosomes could be excluded as possible sites for *neu* by at least two discordances. Moreover, in a mouse × human hybrid containing only the q21-qter region of chromosome 17, the 7.2-kb human *neu* Eco RI segment was also detected (Fig. 2B, lane 4). We conclude that the human *neu* gene is located on q21 of chromosome 17, in contrast to the gene encoding the EGF-r, which is found on chromosome 7.

The localization of *neu* coincides with the map position of the human c-*erb*A1 locus at 17p11-q21 (*13*). The cellular *erb*A and *erb*B genes of chicken have both been incorporated into the genome of avian erythroblastosis virus (*14*). The significance of the coincidental mapping of the *erb*-related *neu* and c-*erb*A1 genes is obscure at present.

While the *neu* and EGF-r genes are distinct and unlinked, they are closely related in at least one region, that which encodes the tyrosine kinase domain. Thus, the *neu* gene, like *erb*B, is a member of the family of genes encoding tyrosine kinase domains. Moreover, the present data suggest that *neu* and *erb*B are more closely related to one another than to other members of this family. Because of the strong similarity in structure of the *neu*- and c-*erb*B-encoded proteins, we believe that the p185 protein, like its relative, is a receptor for a cellular growth factor and mediates similar biological effects.

Although an activated *neu* gene has been isolated from rat neuroglioblastomas, it is noteworthy that the human *neu* gene maps to a chromosomal band that is often involved in a nonrandom reciprocal translocation t(15;17)(q22:q21) in acute promyelocytic leukemia (*15*). The relationship between *neu* and human malignancy is under investigation.

References and Notes

1. C. Shih, L. Padhy, M. Murray, R. A. Weinberg, *Nature (London)* **290**, 261 (1981).
2. A. L. Schechter *et al.*, *ibid.* **312**, 513 (1984).
3. J. Downward *et al.*, *ibid.* **307**, 521 (1984).
4. A. Ullrich *et al.*, *ibid.* **309**, 418 (1984).
5. B. Vennstrom, L. Fanshier, C. Moscovici, J. M. Bishop, *J. Virol.* **36**, 575 (1980).
6. T. Hunter, *Nature (London)* **311**, 414 (1984).
7. M. Basu *et al.*, *ibid.*, p. 477.
8. Y.-H. Xu *et al.*, *ibid.* **309**, 806 (1984).
9. J. Downward, P. Parker, M. D. Waterfield, *ibid.* **310**, 483 (1984).
10. C. R. Lin *et al.*, *Science* **224**, 843 (1984).
11. G. T. Merlino *et al.*, *ibid.*, p. 417.
12. P. Meerakkhan and M. Smith, *Cytogenet. Cell Genet.* **37**, 71 (1984).
13. N. Spurr *et al.*, *EMBO J.* **3**, 159 (1984).
14. B. Vennstrom and J. M. Bishop, *Cell* **28**, 135 (1982).
15. F. Mitelman, *Cytogenet. Cell Genet.* **36**, 1 (1984).
16. U. Francke *et al.*, *Somat. Cell Genet.* **2**, 27 (1976); U. Francke and M. A. Pellegrino, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1147 (1977); U. Francke and B. Francke, *Somat. Cell Genet.* **7**, 71 (1981); U. Francke and B. de Martinville, *Banbury Report* **14**, 175 (1983).
17. The ideogram of chromosome 17 was taken from "An International System for Human Genetic Nomenclature–High Resolution Banding (1981)," *Cytogenet. Cell Genet.* **31**, 1 (1981).
18. J. J. Yunis, *Science* **191**, 1268 (1976).
19. M. E. Harper and G. N. Saunders, *Chromosoma (Berlin)* **83**, 431 (1981).

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Isolation and Propagation of a Human Enteric Coronavirus

Abstract. *Coronavirus-like particles were found by electron microscopy in stools from infants with necrotizing enterocolitis. Stool samples from these infants as well as control specimens were passaged in cultures of human fetal intestinal organs. Two samples yielded virus-like particles and these have now been passaged 14 times (HEC 14). Gradient-purified HEC 14 strains had typical coronavirus morphology on electron microscopy and contained five major proteins with molecular sizes ranging from 190 to 23 kilodaltons. Infants with necrotizing enterocolitis developed specific antibody to the viral antigens between the acute and convalescent stages of the disease, as shown by examining serum specimens by single radial hemolysis, immunoenzymatic assay, and Western immunoblotting. No cross-reactivity was shown with other coronavirus strains tested, or with the newly isolated viruses of the Breda-Berne group, responsible for calf or horse diarrhea.*

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The genus *Coronavirus* contains medium-sized (80 to 150 nm), rounded or polymorphic particles with club-shaped surface projections and a positive-stranded RNA genome. These viruses are widely distributed among various animal species (*1–5*). Only two coronaviruses, the well-characterized OC43 and 229E strains (*6–8*), have been found to cause disease in humans, both viruses producing the common cold. However, coronavirus strains have been associated with diarrheal diseases in lower animals, and there is evidence that these viruses may be involved in human enteric diseases (*9–12*). Most of the data in support of the latter hypothesis result from electron microscopic observations of coro-