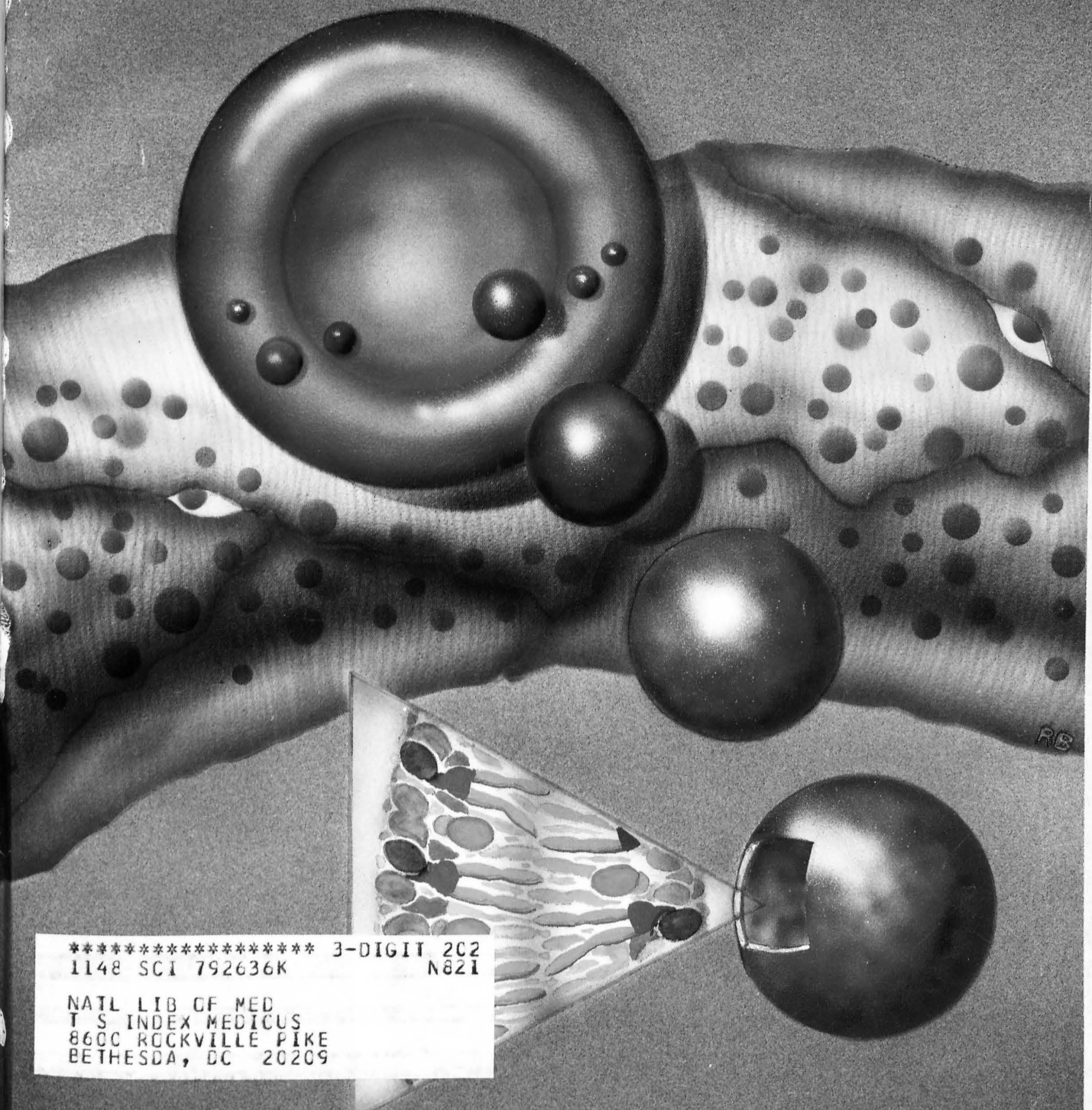


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COVER

Normal red blood cell and smaller hemoglobin-carrying artificial red cells (neohemocytes) set against an isolated capillary. In the capillary, a 25 percent suspension of neohemocytes has replaced the blood. The outer membrane of the neohemocytes, shown enlarged and cutaway, is a bilayer composed of a mixture of four lipids. See page 1165. [Drawing of Robert Burnett, Chartmasters, Inc., San Francisco, California 94133]

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Alzheimer's Disease: A Biologist's Perspectives

Public concerns about Alzheimer's disease are rising. With the increasing survival to advanced ages, it is predicted that Alzheimer's disease will afflict about 2 million people in the United States by the year 2000. Funding for basic and clinical studies on this disease has been increased and now includes \$9 million a year that Congress added to the budget of the National Institute on Aging for ten Alzheimer's disease research centers, about \$40 million from other National Institutes of Health programs, and \$2 million from private foundations. Biologists may ask how the emphasis on Alzheimer's disease could influence support and opportunities for basic research.

My view is that little recognized but implicit aspects of these programs will greatly benefit the neurosciences and biogerontology. An important resource will be the greater availability of brain tissues from normal subjects. To delineate Alzheimer's disease from other common age-related changes requires at least as many (probably several times more) normal controls as individuals with Alzheimer's and other age-related dementias. Postmortem specimens from normal individuals with detailed personal and medical histories are usually scarce. However, healthier relatives and friends of victims of Alzheimer's disease are often willing to donate their own tissues. The Alzheimer's disease research centers could provide the complex logistical support for the short postmortem intervals (4 hours or less) needed to preserve many macromolecules and microscopic structures.

The tissue resources will permit new approaches concerning the impact of heredity and environment on the cellular structure and chemistry of the healthy human brain. The correlation of detailed pre- and postmortem data promises to support major growth of research on human neurobiology and could reveal long-lasting effects of drugs, diet, stress, or even subtler experiences. Pursuit of these far-reaching and difficult questions will also build on the spectacular advances from brain imaging *in vivo*. Other topics so far studied much less in humans than in animals include mechanisms of nonischemic neuronal death; cytoskeletal organization; sex differences; receptors; membrane transport; tissue factors that influence neurite outgrowth; and messenger RNA. The brain messenger RNA's examined at my laboratory and that of M. Morrison have a remarkable postmortem stability; this invites aggressive use of molecular genetic technology.

Screening for hereditary influences on Alzheimer's disease could also reveal genetic markers linked to depression and other common late-onset neurological disorders. Moreover, even without knowing the base sequence of an Alzheimer's locus, linked genetic markers could reveal environmental factors as well as other genes that influence the age of onset and progress of neurological diseases in high-risk individuals.

Studies on Alzheimer's disease also probe basic mechanisms of synaptogenesis. Recently, evidence of neuronal plasticity and sprouting in the human brain was found in the hippocampus of victims of Alzheimer's; these synaptic reorganizations are similar to the changes induced in the rat hippocampus by lesions of the entorhinal cortex.* Intriguing results are being obtained by C. Cotman, F. Gage, D. Gash, and others in the use of embryonic cell transplants to correct experimental or congenital brain lesions that may yield therapies for victims of Alzheimer's. Moreover, research leading to the prevention or effective treatment of Alzheimer's disease seems likely to illuminate one of the great mysteries in biology—the nature of memory and cognition. I would be surprised if the major new resources required for a serious attack on Alzheimer's do not also benefit the basic neurosciences on the same scale as funding for cancer research has done for many areas of molecular, cell, and developmental biology.—CALEB E. FINCH, *Andrus Gerontology Center, Department of Biological Sciences, and Alzheimer Disease Research Center Consortium of Southern California, University of Southern California, Los Angeles 90089*

*J. W. Geddes, D. T. Monaghan, C. W. Cotman, I. T. Lott, R. C. Kim, H. C. Chui, *Science*, this

3000 Ci/mM) (UTP) was then added, and the nuclear suspension was incubated at 30°C for 30 minutes, after which time 15 µl of DNase I (5 µg/ml) in 10 mM CaCl₂ (5 µg/ml) was added. After 5 minutes at 30°C, the reaction was made 1× SET (1 percent sodium dodecyl sulfate (SDS), 5 mM EDTA, 10 mM tris-HCl, pH 7.4), and proteinase K was added to a concentration of 200 µg/ml. After incubation at 37°C for 45 minutes, the solution was extracted with an equal volume of a mixture of phenol and chloroform, and the interphase was again extracted with 100 µl of 1× SET. Ammonium acetate (10M) was added to the combined aqueous phases (original plus reextraction) to a final concentration of 2.3M, an equal volume of isopropyl alcohol was added, and nucleic acid was precipitated (-70°C for 15 minutes). The precipitate was centrifuged in a microcentrifuge for 10 minutes, and the pellet was resuspended in 100 µl of TE (10 mM tris-HCl, 1 mM EDTA) and centrifuged through a G-50 (medium) spin column. The eluate was made 0.2M in NaOH and after 10 minutes on ice, HEPES was added to a concentration of 0.24M. Two and one-half volumes of ethanol were then added, and the solution containing the precipi-

tate held overnight at -20°C. After centrifugation in a microcentrifuge for 5 minutes, the pellet was resuspended in hybridization buffer, which consisted of [10 mM TES, pH 7.4, 0.2 percent SDS, 10 mM EDTA, 0.3M NaCl, 1× Denhardt's, and *Escherichia coli* RNA (250 µg/ml)]. Nitrocellulose filters containing plasmid DNA's were prepared with a Schleicher & Schuell Slot Blot Apparatus under conditions suggested by S and S₂, except that wells were washed with 10× SSC (saline sodium citrate). These filters were first hybridized in the hybridization solution described above for a minimum of 2 hours at 65°C. After this preliminary hybridization, the filters were hybridized to the runoff products in hybridization solution for 36 hours. A typical reaction contained 2 ml of hybridization solution with 1 × 10⁷ cpm/ml. After hybridization, filters were washed for 1 hour in 2× SSC at 65°C. The filters were then incubated at 37°C in 2× SSC with RNase A (10 mg/ml) for 30 minutes and were subsequently washed in 2× SSC at 37°C for 1 hour. Alternatively, after hybridization the filters were washed twice for 15 minutes in 0.1 percent SDS, 2× SSC at room temperature, and then washed at 60°C (0.1 percent SDS, 0.1×

SSC) for 30 minutes. Either protocol for processing of the filters after hybridization yielded the same specificity in signal. Filters were then exposed to Kodak XAR film in cassettes containing Lightening-Plus screens at -70°C for various times.

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48. We thank many of our colleagues for discussion and suggestions during the course of this work; Hal Weintraub, Paul Neiman, and Craig Thompson for comments on the manuscript; Craig Thompson for assistance in obtaining lymphocyte preparations; Bill Schubach for plasmid pBK25; and Kay Shiozaki for assistance with the manuscript. Supported by NIH grants CA 18282 (M.L.) and CA 28151 (M.L. and M.G.), and NSF grant PCM 82-04696 (M.G.), and a scholarship from the Leukemia Society of America (M.G.)

30 July 1985; accepted 15 October 1985

RESEARCH ARTICLE

Tyrosine Kinase Receptor with Extensive Homology to EGF Receptor Shares Chromosomal Location with *neu* Oncogene

Lisa Coussens, Teresa L. Yang-Feng, Yu-Cheng Liao
Ellson Chen, Alane Gray, John McGrath, Peter H. Seeburg
Towia A. Libermann, Joseph Schlessinger, Uta Francke
Arthur Levinson, Axel Ullrich

Growth factors and their receptors are involved in the regulation of cell proliferation, and several recent findings suggest that they also play a key role in oncogenesis (1-4). Of approximately 20 identified oncogenes, the three that have been correlated with known cellular proteins are each related to either a growth factor or a growth factor receptor. The B chain of platelet-derived growth factor (PDGF) is encoded by the proto-oncogene *c-sis* (2), the *erb-B* oncogene product gp68 is a truncated form of the epidermal growth factor (EGF) receptor (3), and the proto-oncogene *c-fms* may be related or identical to the receptor for macrophage colony-stimulating factor (CSF-1^R) (4).

The receptor-related oncogenes are members of a gene family in that each has tyrosine-specific protein kinase activity, and is associated with the plasma membrane (5). Such features are also shared by several other polypeptide hormone receptors, including those for insu-

lin (6), PDGF (7), and insulin-like growth factor I (IGF-1) (8); hence more connections may be found between tyrosine kinase growth factor receptors and tyrosine kinase oncogene products.

Comparison of the complete primary structure of the human EGF receptor (9) with the sequence of the avian erythroblastosis virus (AEV) transforming gene, *v-erbB* (10), revealed close sequence similarity; in addition, there were amino and carboxyl terminal deletions that may reflect key structural changes in the generation of an oncogene from the gene for a normal growth factor receptor (3, 9). Another oncogene, termed *neu*, is also related to *v-erbB* and was originally identified by its activation in ethylnitrosourea-induced rat neuroblastomas (11).

In contrast to *v-erbB*, which encodes a 68,000-dalton truncated EGF receptor, the *neu* oncogene product is a 185,000-dalton cell surface antigen that can be detected by cross-reaction with polyclonal antibodies against EGF receptor (11); *neu* may itself be a structurally altered cell surface receptor with homology to the EGF receptor and binding specificity for an unidentified ligand.

Using *v-erbB* as a screening probe, we isolated genomic and cDNA clones coding for an EGF receptor-related, but distinct, 138,000-dalton polypeptide having all the structural features of a cell surface receptor molecule. On the basis of its structural homology, this putative receptor is a new member of the tyrosine-specific protein kinase family. It is encoded by a 4.8-kb messenger RNA (mRNA) that is widely expressed in normal and malignant tissues. We have localized the gene for this protein to q21 of chromosome 17, which is distinct from the EGF receptor locus, but coincident with the *neu* oncogene mapping position (12). We therefore consider the possibility that we have isolated and characterized the normal human counterpart of the rat *neu* oncogene.

Tyrosine kinase-type receptor gene and complementary DNA. As part of our attempts to isolate and characterize the chromosomal gene coding for the human cellular homologue of the viral *erbB* gp68 polypeptide, AEV-ES4 *erbB* sequences (2.5-kb Pvu II fragment of pAEV) (13) were used as a ³²P-labeled hybridization probe for the screening of a human genomic DNA library at reduced stringency

Lisa Coussens, Yu-Cheng Liao, Ellson Chen, Alane Gray, Peter H. Seeburg, Arthur Levinson, and Axel Ullrich are in the Department of Molecular Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080; John McGrath is currently with the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142; Towia Libermann and Joseph Schlessinger are in the Department of Chemical Immunology at the Weizmann Institute of Science, Rehovot 76100, Israel; and Teresa L. Yang-Feng and Uta Francke are in the Department of Human Genetics at Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510.

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