

SCIENCE LIBRARY

VOLUME 6 • MARCH 1986 • NUMBER 3

LIBRARY

MAR 10 1986

UNIVERSITY OF

Molecular and Cellular Biology



Published monthly by the
American Society for Microbiology

MOLECULAR AND CELLULAR BIOLOGY

VOLUME 6 • MARCH 1986 • NUMBER 3

Aaron J. Shatkin, *Editor in Chief* (1990)
Roche Institute of Molecular Biology
Nutley, N.J.

Harvey F. Lodish, *Editor* (1986)
Whitehead Institute for Biomedical
Research
Cambridge, Mass.

David J. L. Luck, *Editor* (1987)
Rockefeller University
New York, N.Y.

Louis Siminovich, *Editor* (1990)
Mount Sinai Hospital
Toronto, Canada

Joan A. Steitz, *Editor* (1990)
Yale University
New Haven, Conn.

Paul S. Sypherd, *Editor* (1990)
University of California
Irvine

Harold E. Varmus, *Editor* (1989)
University of California
San Francisco

EDITORIAL BOARD

Frederick W. Alt (1987)
Arnold J. Berk (1988)
Alan Bernstein (1987)
Barbara K. Birshtein (1987)
J. Michael Bishop (1987)
Michael R. Botchan (1987)
David Botstein (1987)
Bruce P. Brandhorst (1987)
James R. Broach (1988)
Joan Brugge (1988)
Mario R. Capecchi (1987)
John A. Carbon (1987)
Lawrence A. Chasin (1988)
Nam-Hai Chua (1988)
Don W. Cleveland (1987)
Christopher Coleclough (1987)
Terrance G. Cooper (1987)
Elizabeth A. Craig (1988)
James E. Dahlberg (1987)
James E. Darnell, Jr. (1988)
G. N. Godson (1987)
Michael Green (1988)

Jack F. Greenblatt (1988)
Leonard P. Guarente (1988)
James E. Haber (1987)
Hidesaburo Hanafusa (1986)
Ari Helenius (1987)
Ira Herskowitz (1987)
James B. Hicks (1986)
Alan Hinnebusch (1988)
Michael J. Holland (1987)
Greg Hollis (1987)
Anita K. Hopper (1988)
Peter M. Howley (1988)
Tony Hunter (1986)
Larry Kedes (1988)
Robert S. Kerbel (1988)
Barbara Knowles (1986)
Marilyn Kozak (1988)
Monty Krieger (1986)
Arnold J. Levine (1987)
Susan Lindquist (1987)
Paul T. Magee (1988)
James Manley (1986)

Steven McKnight (1986)
Janet E. Mertz (1987)
Robert L. Metzberg (1988)
Robert K. Mortimer (1988)
Paul Neiman (1986)
Joseph R. Nevins (1987)
Carol Newlon (1988)
Harvey L. Ozer (1988)
Mary Lou Pardue (1988)
Carl S. Parker (1987)
Ira H. Pastan (1988)
David Patterson (1988)
Gianni Piperno (1988)
John R. Pringle (1988)
Jean-Paul Revel (1988)
Daniel B. Rifkin (1988)
G. S. Roeder (1988)
Robert G. Roeder (1988)
Naomi E. Rosenberg (1988)
Norman P. Salzman (1988)
Paul Schedl (1987)
Randy W. Schekman (1988)

Milton J. Schlesinger (1986)
Fred Sherman (1988)
Anna Marie Skalka (1986)
Arthur Skoultschi (1988)
Frank Solomon (1988)
Pamela Stanley (1988)
Bruce Stillman (1988)
Bill Sugden (1988)
Jack Szostak (1987)
Lawrence H. Thompson (1988)
Shirley M. Tilghman (1987)
Robert Tjian (1987)
Jonathan R. Warner (1987)
Alan M. Weiner (1987)
Harold Weintraub (1988)
Reed B. Wickner (1988)
Fred Winston (1988)
Owen Witte (1988)
Elton T. Young (1988)
Michael Young (1988)
Edward Ziff (1988)

Helen R. Whiteley, *Chairman, Publications Board*

Linda M. Illig, *Managing Editor, Journals*

Linda M. Illig, *Production Editor*

Molecular and Cellular Biology (ISSN 0270-7306) is devoted to the advancement and dissemination of fundamental knowledge concerning the molecular biology of eucaryotic cells, of both microbial and higher organisms. Instructions to authors are published in the January issue each year; reprints are available from the editors and the Publications Department. The journal is published monthly, one volume per year. The nonmember subscription price is \$150 per year; single copies are \$21. The member subscription price is \$41 (foreign \$54 [surface rate]) per year; single copies are \$7. Correspondence relating to subscriptions, reprints, defective copies, availability of back issues, lost or late proofs, disposition of submitted manuscripts, and general editorial matters should be directed to the ASM Publications Department, 1913 I St., NW, Washington, DC 20006 (area 202 833-9680).

Claims for missing issues from residents of the United States, Canada, and Mexico must be submitted within 3 months after publication of the issues; residents of all other countries must submit claims within 6 months of publication of the issues. Claims for issues missing because of failure to report an address change or for issues "missing from files" will not be allowed.

Second-class postage paid at Washington, DC 20006, and at additional mailing offices.

POSTMASTER: Send address changes to *Molecular and Cellular Biology*, ASM, 1913 I St., NW, Washington, DC 20006.

Made in the United States of America.

Copyright © 1986, American Society for Microbiology.

All Rights Reserved.

日本：価格は外貨表示とは関係なく円建。

The code at the top of the first page of an article in this journal indicates the copyright owner's consent that copies of the article may be made for personal use or for personal use of specific clients. This consent is given on the condition, however, that the copier pay the stated per-copy fee through the Copyright Clearance Center Inc., 21 Congress St., Salem, MA 01970, for copying beyond that permitted by Sections 107 and 108 of the U.S. Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale.

Localization of a Novel *v-erbB*-Related Gene, *c-erbB-2*, on Human Chromosome 17 and Its Amplification in a Gastric Cancer Cell Line

SHIN-ICHI FUKUSHIGE,¹ KEN-ICHI MATSUBARA,¹ MICHIMIRO YOSHIDA,² MOTOMICHI SASAKI,²
TOSHIMITSU SUZUKI,³ KENTARO SEMBA,⁴ KUMAO TOYOSHIMA,⁴ AND TADASHI YAMAMOTO^{4*}

Institute for Molecular and Cellular Biology, Osaka University, Suita, Osaka 565,¹ Chromosome Research Unit, Hokkaido University, N. 10-W.8, Sapporo 060,² School of Medicine, Niigata University, Niigata 951,³ and The Institute of Medical Science, The University of Tokyo, Minato-ku Tokyo 108,⁴ Japan

Received 17 September 1985/Accepted 3 December 1985

The *c-erbB-2* gene is a *v-erbB*-related proto-oncogene which is distinct from the gene encoding the epidermal growth factor receptor. By using two independent methods, hybridization of both sorted chromosomes and metaphase spreads with cloned *c-erbB-2* DNA, we mapped the *c-erbB-2* locus on human chromosome 17 at q21, a specific breakpoint observed in a translocation associated with acute promyelocytic leukemia. Furthermore, we observed amplification and elevated expression of the *c-erbB-2* gene in the MKN-7 gastric cancer cell line. These data suggest possible involvement of the *c-erbB-2* gene in human cancer.

A number of cellular counterparts to the retroviral oncogenes have been identified and localized on specific chromosomes. The locations of several cellular oncogenes correspond well to breakpoints of chromosomal translocations found in various cancers. For example, the *c-myc* gene on chromosome 8 is involved in translocations between chromosome 8 and one of the chromosomes—2, 14, or 22—that carries an immunoglobulin gene (5, 9, 12, 22). The resulting alteration in *c-myc* expression is suspected to be causally related to tumorigenesis (14).

An avian erythroblastosis virus H strain contains an oncogene, *v-erbB*, that replaces the *env* gene of an avian leukemia virus (25). The nucleotide sequence analysis of the cloned *v-erbB* DNA and human epidermal growth factor (EGF) receptor cDNA clones revealed that the *v-erbB* protein corresponds to the carboxyl half of the human EGF receptor, including the membrane-spanning domain (23, 24, 26). This strongly suggests that the 3' half of the chicken EGF receptor gene was transduced into the H strain of avian erythroblastosis virus. In addition to the EGF receptor gene, we found another *v-erbB*-related gene, *c-erbB-2*, in the human genome. The *c-erbB-2* gene is apparently distinct from the EGF receptor gene, since transcripts of the two genes differ from each other in length and because the amino acid sequence predicted from the nucleotide sequence of cloned *c-erbB-2* gene is very similar to the corresponding region of the EGF receptor (17). Recently, the *neu* oncogene, active in a series of rat neuroblastoma (19), was found to be an *erbB*-related gene encoding an EGF receptor-like protein (C. I. Bargmann, M.-C. Huang, and R. A. Weinberg, *Nature* [London], in press). Comparison of the nucleotide sequences and the deduced amino acid sequences of human *c-erbB-2* (T. Yamamoto, S. Ikawa, T. Akiyama, K. Semba, N. Nomura, N. Miyajima, T. Saito, and K. Toyoshima, *Nature* [London], in press) and rat *neu* (Bargmann et al., in press) revealed a strong similarity between the two genes, which suggests that they are in fact the same gene.

Metaphase chromosomes were prepared from two cell lines, GM2324 and GM3197, which were provided by the

Human Genetic Mutant Cell Repository and then sorted (Institute for Medical Research, Camden, N.J.), into nine fractions using a fluorescence-activated cell sorter as described previously (20, 28). DNA samples were prepared from each fraction of the sorted chromosomes (7) and analyzed by Southern hybridization (21) using a DNA probe of a 440-base-pair (bp) *KpnI-XbaI* restriction fragment (KX-DNA) generated from the *c-erbB-2* genomic clone λ 107. With the GM2324 cell line, a positive signal was observed for a fraction that corresponded mainly to chromosomes 16, 17, and 18 (data not shown). Another human lymphoblast culture, GM3197, carries the reciprocal translocation, t(17;22), producing derivative chromosomes (17:22 and 22q⁻) that are different in size from the normal homologs (6). Analysis of this cell line revealed two positive signals, one in a fraction that contains normal chromosome 17 and one in a fraction that contains the derivative chromosome 17:22 (data not shown). These results indicate that the *c-erbB-2* gene is located on human chromosome 17.

To localize the *c-erbB-2* gene more precisely, we performed in situ hybridization experiments on chromosome spreads prepared from phytohemagglutinin-stimulated peripheral blood cultures (3, 29). The probe used for this experiment was ³H-labeled pCER217 plasmid DNA, which is a *c-erbB-2* cDNA clone containing a 2.7-kbp insert in the Okayama-Berg vector (15). Analysis of 85 metaphase cells revealed that 23.5% (20 of 85) of the silver grains were located on chromosome 17. Of these 20 grains, 15 (75%) were located on band q21-q22, and 11 grains in particular were in the region 17q21.1-21.3 (Fig. 1). A human version of the *neu* oncogene was recently mapped on human chromosome 17 at q21 (16). Since translocation between chromosomes 15 and 17, t(15;17) (q23;q21), is associated with acute promyelocytic leukemia (APL) (13), we examined DNA from seven cases of APL for the possible involvement of the *c-erbB-2* gene in this leukemia. Using the ³²P-labeled fragment prepared from pCER217 as a probe, we observed no sign of rearrangement of the *c-erbB-2* gene by Southern hybridization analysis (data not shown). Recently, the *p53* gene was also mapped to human chromosome 17 at bands 17q21-q22. Although rearrangements of the *p53* gene were not observed on Southern blotting of DNAs from APL cells

* Corresponding author.



FIG. 1. Localization of the *c-erbB-2* gene by in situ hybridization. (a) Photograph of a lymphocyte metaphase spread hybridized with the *c-erbB-2* probe nick translated with [^3H]dCTP (30 Ci/mmol) and [^3H]dTTP (48 Ci/mmol). The specific activity of the probe was 3×10^7 cpm/ μg of DNA. The chromosomal DNA was denatured on slides in 70% formamide- $2\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 70°C for 2 min and then hybridized in a solution of 50% formamide- $2\times$ SSC-40 mM sodium phosphate (pH 7.0)-10% dextran sulfate-denatured salmon sperm DNA (100 $\mu\text{g}/\text{ml}$)- $1\times$ Denhardt solution for 16 h at 40°C . After hybridization, the slides were rinsed for 10 min twice in 50% formamide- $2\times$ SSC at 40°C and then several times in $0.2\times$ SSC at 37°C . Autoradiography was performed using half-strength Sakura NR-M2 emulsion (Konishiroku, Tokyo) for 3 weeks at 4°C . Chromosomes were Q banded using the double-staining method with quinacrine-mustard and Hoechst 33258 (27) and analyzed under a fluorescence microscope (left). Silver grains were detected by visible light and were identified on Q-banded chromosomes (right). (b) Distribution of 20 grains over chromosome 17.

with t(15;17), translocation of the *p53* gene to chromosome 15 was observed in three of three APL cases tested (8) by in situ hybridization. Thus, further analysis of APL cells with t(15;17), which include in situ hybridization on chromosome

spread using *c-erbB-2*-specific DNA probes, is anticipated.

Previously, we found that the *c-erbB-2* gene is amplified in an adenocarcinoma of the salivary gland, although we could

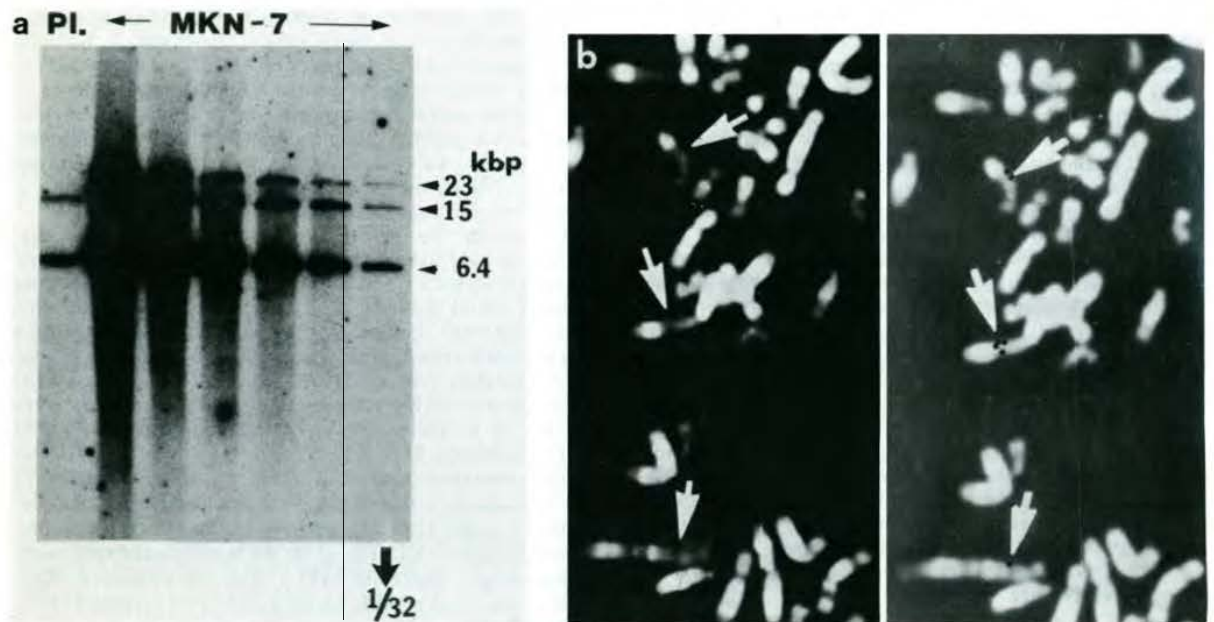


FIG. 2. Amplification and translocation of the *c-erbB-2* gene in a gastric cancer cell line, MKN-7. (a) Amplification of the *c-erbB-2* gene. High-molecular-weight DNAs were prepared from MKN-7 and human placental cells and digested with restriction endonuclease *EcoRI*. A nitrocellulose filter containing the *EcoRI* digests was probed with [^{32}P]labeled KX-DNA (specific activity; 10^8 cpm/ μg of DNA). Hybridization was carried out in a stringent condition (17). The filter contained, in lanes from left to right, placental DNA (10 μg) and MKN-7 DNA (10, 5, 2.5, 1.25, 5/8, and 5/16 μg). (b) Translocation of the *c-erbB-2* gene. Metaphase spread was prepared from MKN-7 cells (left) and hybridized with [^3H]labeled pCER217 DNA (right) as described in the legend to Fig. 1. Arrows indicate location of the *c-erbB-2* gene on marker chromosomes.

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.