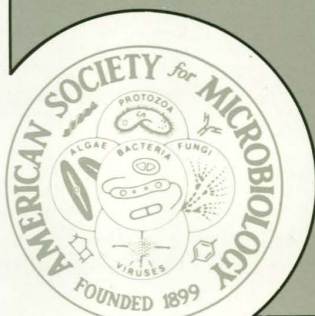


VOLUME 9 • **MARCH 1989** • NUMBER 3

Molecular and Cellular Biology



**Published monthly by the
American Society for Microbiology**

CODEN: MCEBD4

9 (3) 875-1380 (1989)

ISSN: 0270-7306

BIOEPIS EX. 1021

Page 1

MOLECULAR AND CELLULAR BIOLOGY

VOLUME 9 • MARCH 1989 • NUMBER 3

Aaron J. Shatkin, *Editor in Chief* (1990)
*Center for Advanced
Biotechnology and Medicine
Piscataway, N.J.*

Tony Hunter, *Editor* (1993)
*The Salk Institute
San Diego, Calif.*

David J. L. Luck, *Editor* (1992)
*Rockefeller University
New York, N.Y.*

Steven L. McKnight, *Editor* (1992)
*Carnegie Institution of Washington
Baltimore, Md.*

Randy W. Schekman, *Editor*
(1992)
*University of California
Berkeley*

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

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

Shirley M. Tilghman, *Editor* (1993)
*Princeton University
Princeton, N.J.*

Robert Tjian, *Editor* (1991)
*University of California
Berkeley*

Alan M. Weiner, *Editor* (1993)
*Yale University
New Haven, Conn.*

Owen N. Witte, *Editor* (1993)
*Molecular Biology Institute
University of California
Los Angeles*

EDITORIAL BOARD

Frederick W. Alt (1990)
Susan Berget (1990)
Arnold J. Berk (1991)
Alan Bernstein (1990)
Barbara K. Birshtein (1990)
J. Michael Bishop (1990)
Elizabeth H. Blackburn
(1991)
Jef D. Boeke (1991)
Michael R. Botchan (1990)
David Botstein (1990)
Bruce P. Brandhorst (1990)
Marjorie C. Brandriss
(1991)
Kathryn Calame (1991)
Mario R. Capecchi (1990)
John A. Carbon (1990)
Marian Carlson (1989)
C. Thomas Caskey (1990)
Lawrence A. Chasin (1991)
Nam-Hai Chua (1991)
Don W. Cleveland (1990)
Nicholas Cowan (1990)
Elizabeth A. Craig (1991)
Thomas Curran (1991)
Mark Davis (1990)
Gideon Dreyfuss (1990)

Mary P. Edmonds (1990)
Robert N. Eisenman (1991)
Sarah C. R. Elgin (1991)
Scott Emr (1991)
Walton L. Fangman (1991)
Thomas D. Fox (1990)
Mary-Jane Gething (1990)
Steve Goff (1989)
Jack F. Greenblatt (1991)
Leonard P. Guarente (1991)
Christine Guthrie (1989)
James E. Haber (1990)
Hidesaburo Hanafusa (1989)
Leland D. Hartwell (1990)
Nathaniel Heintz (1991)
Ari Helenius (1990)
Steven Henikoff (1991)
Ira Herskowitz (1990)
James B. Hicks (1989)
Alan Hinnebusch (1991)
Michael J. Holland (1990)
Greg Hollis (1990)
Anita K. Hopper (1991)
Peter M. Howley (1991)
Mark Johnson (1991)
Daniel Klessig (1989)
Barbara Knowles (1989)

Marilyn Kozak (1991)
Monty Krieger (1989)
Elizabeth Lacy (1990)
Alan Lambowitz (1990)
Michael Lenardo (1991)
Arthur D. Levinson (1990)
Susan Lindquist (1990)
Stuart M. Linn (1989)
Douglas Lowy (1990)
Paul T. Magee (1991)
James Manley (1989)
Kunihiro Matsumoto (1991)
Janet E. Mertz (1990)
Bernardo Nadal-Ginard (1990)
Paul Neiman (1989)
Joseph R. Nevins (1990)
Carol Newlon (1991)
Mary Ann Osley (1990)
Harvey L. Ozer (1991)
Mary Lou Pardue (1991)
David Patterson (1991)
Gianni Piperno (1991)
John R. Pringle (1991)
Steven I. Reed (1991)
Jean-Paul Revel (1991)
Jasper D. Rine (1991)
Naomi E. Rosenberg (1991)

Rodney Rothstein (1990)
Norman P. Salzman (1991)
Paul Schedl (1990)
Milton J. Schlesinger (1989)
Matthew P. Scott (1989)
Charles J. Sherr (1991)
Arthur Skoultschi (1991)
Barbara Sollner-Webb (1989)
Frank Solomon (1991)
Michael B. Sporn (1991)
Allan C. Spradling (1991)
Karen Sprague (1989)
Pamela Stanley (1991)
Nat Sternberg (1989)
Bruce Stillman (1991)
Kevin Struhl (1989)
Kenneth D. Stuart (1991)
Bill Sugden (1991)
Lawrence H. Thompson (1991)
Geoffrey Wahl (1989)
Peter Walter (1990)
Jonathan R. Warner (1990)
Reed B. Wickner (1991)
Lewis T. Williams (1991)
Fred Winston (1991)
Elton T. Young (1990)
Michael Young (1991)

Helen R. Whiteley, *Chairman, Publications Board*
Linda M. Illig, *Managing Editor, Journals*

Kirk Jensen, *Director of Publications*
Linda M. Illig, *Production Editor*

Molecular and Cellular Biology (ISSN 0270-7306), a publication of the American Society for Microbiology, 1913 I St., N.W., Washington, DC 20006-5107, 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 \$340 per year; single copies are \$30. The member subscription price is \$43 (foreign, \$71 [air drop shipping]) per year; single copies are \$8. 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., N.W., Washington, DC 20006-5107 (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., N. W., Washington, DC 20006-5107.

Made in the United States of America. Printed on acid-free paper.
Copyright © 1989, 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.

Author Index

- Agabian, Nina, 1212
 Alitalo, Kari, 1255
 Arceci, Robert J., 1346
- Barringer, Kevin J., 1316
 Bedard, Pierre-Andre, 1371
 Bedwell, David M., 1014
 Beemon, Karen, 1155
 Bernstein, Mitchell, 1191
 Bertrand, Helmut, 1362
 Birshstein, Barbara K., 1324
 Bishop, J. Michael, 1148
 Bissinger, Peter H., 1309
 Bloom, Kerry, 1368
 Bos, Timothy J., 1255
 Brownlee, Clare, 1060
 Brugge, Joan S., 1109
 Burke, Dan, 1049
- Calos, Michele P., 1026
 Caratoli, Alessandra, 1271
 Carle, George S., 983
 Chae, Chi-Bom, 1005
 Chalkley, Roger, 1289
 Challoner, Peter B., 902
 Chedid, Marcio, 959
 Chin, William W., 1128
 Clayton, Christine E., 1332
 Clayton, David A., 1200
 Conscience, Jean-François, 1183
 Cregg, James M., 1316
 Croop, James M., 1346
 Culotta, Valeria Cizewski, 1376
- Darling, Douglas S., 1128
 Davies, Monique V., 946, 1233
 DeMarco, Michael, 1109
 Deuring, Renate, 875
 Deutsch, Walter A., 965
 Devault, Alain, 1346
 Diamantis, Ioannis D., 1183
 Didion, Thomas, 988
 Dihanich, Melitta, 1100
 Dorner, Andrew J., 1233
 Dubin, Robert A., 1083
- Ehrlich, Kenneth C., 1351
 Ehrlich, Melanie, 1351
 Elliott, David J., 1069
 Emr, Scott D., 1014
 Erikson, R. L., 1371
- Felsenfeld, Gary, 893
 Fendly, Brian M., 1165
 Fisch, Tobe M., 1327
 Fluck, Michele M., 995
 Foreman, Pamela K., 1137
 Fox, Larry G., 1109
 Frankel, Wayne, 1284
 Fu, Ying-Hui, 1120
 Fuller, Margaret T., 875
- Garcia-Blanco, Mariano A., 1060
 Gasdaska, Pam, 1049
 Ghazal, Peter, 1342
 Granner, Daryl K., 1289
 Groen, N. A., 1277
 Gros, Philippe, 1346
 Groudine, Mark, 902
- Haase, Steven B., 1026
 Haber, Daniel, 1346
 Hacker, David, 995
 Hamer, Dean H., 1376
 Hamilton, Barbara, 1309
 Hamlin, Joyce L., 1137
 Hanahan, Douglas, 925
 Hannigan, Gregory, 1060
 Harless, Julie, 965
 Hartwell, Leland, 1049
 Hays, Thomas S., 875
 Hennighausen, Lothar, 1342
 Hershey, John W. B., 946
 Hill, Alison, 1368
 Hodin, Richard A., 1128
 Hofer, Paul, 1183
 Holland, Janice P., 1243
 Holland, Michael J., 1243
 Housman, David E., 1346
 Howley, Peter M., 925
 Hudziak, Robert M., 1165
 Hwang, Inhwan, 1005
- Ip, Y. Tony, 1289
 Irving, Steven G., 1034, 1041
 Israel, David I., 1233
- Jacobs, Howard T., 1069
 Jerry, D. Joseph, 935
 Johnston, Leslie A., 935
 Jones, Peter A., 885
 Jongeward, Gregg D., 1014
 June, Carl H., 1034
- Kang, John, 1243
 Katzenberg, Daniel R., 1324
 Kaufman, Randal J., 946, 1233
 Kelley, Mark R., 965
 Kelly, Kathleen, 1034, 1041
 Kepes, François, 1191
 Keski-Oja, Jorma, 1255
 Kim, Ki-Han, 974
 Kindler, Vincent, 1183
 Knezetic, Joseph A., 893
 Kobayashi, George S., 983
 Kotarski, Michael A., 935
 Kowalik, Klaus V., 988
 Kozak, Leslie P., 935
 Krag, Sharon S., 914
 Krysan, Patrick J., 1026
- Lambowitz, Alan M., 1362
 Lambris, John D., 1100
 Lazar, Mitchell A., 1128
- Lengyel, Peter, 1060
 Lewis, Gail D., 1165
 Ling, Victor, 1224
 Lizardi, Paul M., 1212
 Lockshin, Curtis, 1342
 Lohman, P. H. M., 1277
 Lowenhaupt, Ky, 1173
 Lubon, Henryk, 1342
 Lugo, Tracy Gross, 1263
 Lührmann, Reinhard, 1212
- Macino, Giuseppe, 1271
 Madden, Knut R., 1316
 Mannix, Daniel G., 1120
 Marshallsay, Brigitte, 1100
 Marzluf, George A., 1120
 McIntosh, Lee, 1362
 Medoff, Gerald, 983
 Mestel, Rosie, 1243
 Michalowsky, Lesley A., 885
 Mizel, Steven B., 959
 Morelli, Giorgio, 1271
 Moroni, Christoph, 1183
 Morrison, Elizabeth, 1060
 Moss, Stuart B., 902
 Mottram, Jeremy, 1212
 Mowatt, Michael R., 1332
- Nair, Asha P. K., 1183
 Nelson, F. Kenneth, 1284
 Nelson, Jay, 1342
 Nelson, Mary Anne, 1271
 Nelson, Richard G., 1212
 Nemeth, Susan P., 1109
 Neumann, Dieter, 1298
 Ng, William F., 1224
 Nickels, Roxy, 1362
 Nover, Lutz, 1298
 Nusse, Roel, 1357
- Paietta, John V., 1120
 Pape, Michael E., 974
 Pardue, Mary Lou, 1173
 Pathak, Vinay K., 946
 Perry, Karen L., 1212
 Pertovaara, Liisa, 1255
 Piatigorsky, Joram, 1083
 Pollok, Brian A., 959
 Prout, Mary, 875
 Prywes, Ron, 1327
- Rajan, T. V., 1284
 Ray, Dan S., 1365
 Raymond, Martine, 1346
 Reynolds-Kohler, Catherine, 1342
 Rich, Alexander, 1173
 Robertson, Barbara, 875
 Roeder, Robert G., 1327
 Roelink, Henk, 1357
 Roggenkamp, Rainer, 988
 Romano, Nicoletta, 1271
 Rosenwald, Anne G., 914
 Ruis, Helmut, 1309
- Rutherford, Michael, 1060
 Ryden, Thomas A., 1155
- Sabourin, Josanne R., 1362
 Sarangi, Farida, 1224
 Scharf, Klaus-Dieter, 1298
 Schekman, Randy, 1191
 Schuurin, Ed, 1357
 Shepard, H. Michael, 1165
 Shirakawa, Fumihiko, 959
 Shtivelman, E., 1148
 Siebenlist, Ulrich, 1034, 1041
 Simmons, Daniel L., 1371
 Simons, J. W. I. M., 1277
 Sippola-Thiele, Maria, 925
 Sistonen, Lea, 1255
 Stanley, Pamela, 914
 Steele, Paul E., 983
 Stiles, Charles D., 1060
 Stillman, Cathy A., 1316
 Strobel, Scott A., 1014
 Supakar, Prakash C., 1351
 Suttles, Jill, 959
- Thill, Gregory P., 1316
 Tilley, Shermaine A., 1324
 Topper, James N., 1200
- Ullrich, Axel, 1165
- van Deemter, Liesbeth, 1357
 van Rooijen, M. L., 1277
 Van Tuinen, Evert, 1100
 van Zeeland, A. A., 1277
 Veinot-Drebot, Lela, 1224
 Venugopal, Sheela, 965
 Verma, Inder M., 1336
 Visvader, Jane, 1336
 Vogt, Peter K., 1255
 Vrieling, H., 1277
- Wang, Enduo, 1243
 Wasley, Louise C., 1233
 Wawrousek, Eric F., 1083
 Wieser, Rotraud, 1309
 Williams, Bryan R. G., 1060
 Winget, Marcy, 1165
 Wisdom, Gregory S., 1332
 Wise, Robert J., 1233
 Witte, Owen N., 1263
- Yannoni, Yvonne, 1371
 Yao, Ching-Ho, 1092
 Yao, Meng-Chao, 1092
 Yip, Michele, 1243
 Yun, Kyuson, 1014
- Zastawny, Roman L., 1224
 Zdzienicka, M. Z., 1277
 Zhang, Daoling, 1351
 Zipfel, Peter F., 1034, 1041

MOLECULAR AND CELLULAR BIOLOGY

Volume 9

March 1989

No. 3

GENE EXPRESSION

Gene Structure and Transcription in Mouse Cells with Extensively Demethylated DNA. Lesley A. Michalowsky and Peter A. Jones.....	885-892
Identification and Characterization of a Chicken α-Globin Enhancer. Joseph A. Knezetic and Gary Felsenfeld.....	893-901
Control of Carbohydrate Processing: Increased β-1,6 Branching in N-Linked Carbohydrates of Lec9 CHO Mutants Appears To Arise from a Defect in Oligosaccharide-Dolichol Biosynthesis. Anne G. Rosenwald, Pamela Stanley, and Sharon S. Krag.....	914-924
An Ubiquitously Expressed Gene 3.5 Kilobases Upstream of the Glycerol-3-Phosphate Dehydrogenase Gene in Mice. Leslie A. Johnston, Michael A. Kotarski, D. Joseph Jerry, and Leslie P. Kozak.....	935-945
The Phosphorylation State of Eucaryotic Initiation Factor 2 Alters Translational Efficiency of Specific mRNAs. Randal J. Kaufman, Monique V. Davies, Vinay K. Pathak, and John W. B. Hershey.....	946-958
Interleukin 1 and Cyclic AMP Induce κ Immunoglobulin Light-Chain Expression via Activation of an NF-κB-Like DNA-Binding Protein. Fumihiko Shirakawa, Marcio Chedid, Jill Suttles, Brian A. Pollok, and Steven B. Mizel.....	959-964
Transcriptional Regulation of Acetyl Coenzyme A Carboxylase Gene Expression by Tumor Necrosis Factor in 30A-5 Preadipocytes. Michael E. Pape and Ki-Han Kim.....	974-982
S-Phase-Specific Transcription Regulatory Elements Are Present in a Replication-Independent Testis-Specific H2B Histone Gene. Inhwon Hwang and Chi-Bom Chae.....	1005-1013
Mitogen-Induced Genes Are Subject to Multiple Pathways of Regulation in the Initial Stages of T-Cell Activation. Steven G. Irving, Carl H. June, Peter F. Zipfel, Ulrich Siebenlist, and Kathleen Kelly.....	1034-1040
Complexity of the Primary Genetic Response to Mitogenic Activation of Human T Cells. Peter F. Zipfel, Steven G. Irving, Kathleen Kelly, and Ulrich Siebenlist.....	1041-1048
Regulation of 2',5'-Oligoadenylate Synthetase Gene Expression by Interferons and Platelet-Derived Growth Factor. Mariano A. Garcia-Blanco, Peter Lengyel, Elizabeth Morrison, Clare Brownlee, Charles D. Stiles, Michael Rutherford, Gregory Hannigan, and Bryan R. G. Williams.....	1060-1068
Mutually Exclusive Synthetic Pathways for Sea Urchin Mitochondrial rRNA and mRNA. David J. Elliott and Howard T. Jacobs.....	1069-1082
Expression of the Murine αB-Crystallin Gene Is Not Restricted to the Lens. Robert A. Dubin, Eric F. Wawrousek, and Joram Piatigorsky.....	1083-1091
<i>cys-3</i>, the Positive-Acting Sulfur Regulatory Gene of <i>Neurospora crassa</i>, Encodes a Protein with a Putative Leucine Zipper DNA-Binding Element. Ying-Hui Fu, John V. Palletta, Daniel G. Mannix, and George A. Marzluf.....	1120-1127
A Novel Member of the Thyroid/Steroid Hormone Receptor Family Is Encoded by the Opposite Strand of the Rat <i>c-erbAα</i> Transcriptional Unit. Mitchell A. Lazar, Richard A. Hodin, Douglas S. Darling, and William W. Chin....	1128-1136

Continued on following page

Continued from preceding page

Avian Retroviral Long Terminal Repeats Bind CCAAT/Enhancer-Binding Protein. Thomas A. Ryden and Karen Beemon	1155-1164
Identification of Transcriptional Regulatory Elements in Human Mitochondrial DNA by Linker Substitution Analysis. James N. Topper and David A. Clayton	1200-1211
Isolation and Sequence of Four Small Nuclear U RNA Genes of <i>Trypanosoma brucei</i> subsp. <i>brucei</i>: Identification of the U2, U4, and U6 RNA Analogs. Jeremy Mottram, Karen L. Perry, Paul M. Lizardi, Reinhard Lührmann, Nina Agabian, and Richard G. Nelson	1212-1223
Effect of von Willebrand Factor Coexpression on the Synthesis and Secretion of Factor VIII in Chinese Hamster Ovary Cells. Randal J. Kaufman, Louise C. Wasley, Monique V. Davies, Robert J. Wise, David I. Israel, and Andrew J. Dorner	1233-1242
Sequences within the Spacer Region of Yeast rRNA Cistrons That Stimulate 35S rRNA Synthesis In Vivo Mediate RNA Polymerase I-Dependent Promoter and Terminator Activities. Rosie Mestel, Michele Yip, Janice P. Holland, Enduo Wang, John Kang, and Michael J. Holland	1243-1254
Enhanced <i>jun</i> Gene Expression Is an Early Genomic Response to Transforming Growth Factor β Stimulation. Liisa Pertovaara, Lea Sistonen, Timothy J. Bos, Peter K. Vogt, Jorma Keski-Oja, and Kari Alitalo	1255-1262
The BCR-ABL Oncogene Transforms Rat-1 Cells and Cooperates with <i>v-myc</i>. Tracy Gross Lugo and Owen N. Witte	1263-1270
Molecular Cloning of a <i>Neurospora crassa</i> Carotenoid Biosynthetic Gene (Albino-3) Regulated by Blue Light and the Products of the White Collar Genes. Mary Anne Nelson, Giorgio Morelli, Alessandra Carattoli, Nicoletta Romano and Giuseppe Macino	1271-1276
Hormonal Regulation of Phosphoenolpyruvate Carboxykinase Gene Expression Is Mediated through Modulation of an Already Disrupted Chromatin Structure. Y. Tony Ip, Daryl K. Granner, and Roger Chalkley	1289-1297
Cytoplasmic Heat Shock Granules Are Formed from Precursor Particles and Are Associated with a Specific Set of mRNAs. Lutz Nover, Klaus-Dieter Scharf, and Dieter Neumann	1298-1308
Functional Characterization of the Two Alcohol Oxidase Genes from the Yeast <i>Pichia pastoris</i>. James M. Cregg, Knut R. Madden, Kevin J. Barringer, Gregory P. Thill, and Cathy A. Stillman	1316-1323
Nucleotide Sequence of an Unequal Sister Chromatid Exchange Site in a Mouse Myeloma Cell Line. Daniel R. Katzenberg, Shermaine A. Tilley, and Barbara K. Birshtein	1324-1326
An AP1-Binding Site in the <i>c-fos</i> Gene Can Mediate Induction by Epidermal Growth Factor and 12-<i>O</i>-Tetradecanoyl Phorbol-13-Acetate. Tobe M. Fisch, Ron Prywes, and Robert G. Roeder	1327-1331
Differential Transcription of Exon 1 of the Human <i>c-fms</i> Gene in Placental Trophoblasts and Monocytes. Jane Visvader and Inder M. Verma	1336-1341
Cell-Specific Activity of the Modulator Region in the Human Cytomegalovirus Major Immediate-Early Gene. Henryk Lubon, Peter Ghazal, Lothar Hennighausen, Catherine Reynolds-Kohler, Curtis Lockshin, and Jay Nelson	1342-1345
The Three Mouse Multidrug Resistance (<i>mdr</i>) Genes Are Expressed in a Tissue-Specific Manner in Normal Mouse Tissues. James M. Croop, Martine Raymond, Daniel Haber, Alain Devault, Robert J. Arceci, Philippe Gros, and David E. Housman	1346-1350

Continued on following page

Continued from preceding page

Rapid Repression of Quiescence-Specific Gene Expression by Epidermal Growth Factor, Insulin, and pp60^{v-src}. Pierre-Andre Bedard, Yvonne Yannoni, Daniel L. Simmons, and R. L. Erikson.....	1371-1375
Fine Mapping of a Mouse Metallothionein Gene Metal Response Element. Valeria Cizewski Culotta and Dean H. Hamer	1376-1380

CELL GROWTH AND DEVELOPMENT

Expression of Replication-Dependent Histone Genes in Avian Spermatids Involves an Alternate Pathway of mRNA 3'-End Formation. Peter B. Challoner, Stuart B. Moss, and Mark Groudine	902-913
Cell-Heritable Stages of Tumor Progression in Transgenic Mice Harboring the Bovine Papillomavirus Type 1 Genome. Maria Sippola-Thiele, Douglas Hanahan, and Peter M. Howley	925-934
Dominant Effects of Tubulin Overexpression in <i>Saccharomyces cerevisiae</i>. Dan Burke, Pam Gasdaska, and Leland Hartwell	1049-1059
Deletions within the Amino-Terminal Half of the <i>c-src</i> Gene Product That Alter the Functional Activity of the Protein. Susan P. Nemeth, Larry G. Fox, Michael DeMarco, and Joan S. Brugge.....	1109-1119
The <i>PVT</i> Gene Frequently Amplifies with <i>MYC</i> in Tumor Cells. E. Shtivelman and J. Michael Bishop.....	1148-1154
p185^{HER2} Monoclonal Antibody Has Antiproliferative Effects In Vitro and Sensitizes Human Breast Tumor Cells to Tumor Necrosis Factor. Robert M. Hudziak, Gail D. Lewis, Marcy Winget, Brian M. Fendly, H. Michael Shepard, and Axel Ullrich	1165-1172
A v-H-<i>ras</i>-Dependent Hemopoietic Tumor Model Involving Progression from a Clonal Stage of Transformation Competence to Autocrine Interleukin 3 Production. Asha P. K. Nair, Ioannis D. Diamantis, Jean-François Conscience, Vincent Kindler, Paul Hofer, and Christoph Moroni.....	1183-1190
Control of <i>Saccharomyces cerevisiae</i> Catalase T Gene (<i>CTTI</i>) Expression by Nutrient Supply via the RAS-Cyclic AMP Pathway. Peter H. Bissinger, Rotraud Wieser, Barbara Hamilton, and Helmut Ruis.....	1309-1315
Variation of Tandem Repeats in the Developmentally Regulated Procylic Acidic Repetitive Proteins of <i>Trypanosoma brucei</i>. Michael R. Mowatt, Gregory S. Wisdom, and Christine E. Clayton.....	1332-1335
Transient Expression of the Proto-Oncogene <i>int-1</i> during Differentiation of P19 Embryonal Carcinoma Cells. Ed Schuurin, Liesbeth van Deemter, Henk Roelink, and Roel Nusse	1357-1361

CELL AND ORGANELLE STRUCTURE AND ASSEMBLY

Interacting Proteins Identified by Genetic Interactions: a Missense Mutation in α-Tubulin Fails To Complement Alleles of the Testis-Specific β-Tubulin Gene of <i>Drosophila melanogaster</i>. Thomas S. Hays, Renate Deuring, Barbara Robertson, Mary Prout, and Margaret T. Fuller	875-884
Formation of Irregular Giant Peroxisomes by Overproduction of the Crystallin Core Protein Methanol Oxidase in the Methylophilic Yeast <i>Hansenula polymorpha</i>. Rainer Roggenkamp, Thomas Didion, and Klaus V. Kowallik	988-994

Continued on following page

Continued from preceding page

Sequence and Structural Requirements of a Mitochondrial Protein Import Signal Defined by Saturation Cassette Mutagenesis. David M. Bedwell, Scott A. Strobel, Kyuson Yun, Gregg D. Jongeward, and Scott D. Emr.....	1014-1025
Accumulation of Viruslike Particles in a Yeast Mutant Lacking a Mitochondrial Pore Protein. Melitta Dihanich, Evert Van Tuinen, John D. Lambris, and Brigitte Marshallsay.....	1100-1108
SEC59 Encodes a Membrane Protein Required for Core Glycosylation in <i>Saccharomyces cerevisiae</i>. Mitchell Bernstein, François Kepes, and Randy Schekman.....	1191-1199
Immunological Identification of the Alternative Oxidase of <i>Neurospora crassa</i> Mitochondria. Alan M. Lambowitz, Josanne R. Sabourin, Helmut Bertrand, Roxy Nickels, and Lee McIntosh.....	1362-1364
Conserved Sequence Blocks in Kinetoplast Minicircles from Diverse Species of Trypanosomes. Dan S. Ray.....	1365-1367

CHROMOSOME STRUCTURE AND DYNAMICS

Antibody to a Human DNA Repair Protein Allows for Cloning of a <i>Drosophila</i> cDNA That Encodes an Apurinic Endonuclease. Mark R. Kelley, Sheela Venugopal, Julie Harless, and Walter A. Deutsch.....	965-973
Electrophoretic Analysis of <i>Histoplasma capsulatum</i> Chromosomal DNA. Paul E. Steele, Georges F. Carle, George S. Kobayashi, and Gerald Medoff.....	983-987
High-Level Recombination Specific to Polyomavirus Genomes Targeted to the Integration-Transformation Pathway. David Hacker and Michele M. Fluck.....	995-1004
Isolation of Human Sequences That Replicate Autonomously in Human Cells. Patrick J. Krysan, Steven B. Haase, and Michele P. Calos.....	1026-1033
Accurate Processing and Amplification of Cloned Germ Line Copies of Ribosomal DNA Injected into Developing Nuclei of <i>Tetrahymena thermophila</i>. Meng-Chao Yao and Ching-Ho Yao.....	1092-1099
Identification and Characterization of a Gene That Is Coamplified with Dihydrofolate Reductase in a Methotrexate-Resistant CHO Cell Line. Pamela K. Foreman and Joyce L. Hamlin.....	1137-1147
Nonrandom Distribution of Long Mono- and Dinucleotide Repeats in <i>Drosophila</i> Chromosomes: Correlations with Dosage Compensation, Heterochromatin, and Recombination. Ky Lowenhaupt, Alexander Rich, and Mary Lou Pardue.....	1173-1182
Identification of Members of the P-Glycoprotein Multigene Family. William F. Ng, Farida Sarangi, Roman L. Zastawny, Lela Veinot-Drebot, and Victor Ling.....	1224-1232
DNA Strand Specificity for UV-Induced Mutations in Mammalian Cells. H. Vrieling, M. L. van Rooijen, N. A. Groen, M. Z. Zdzienicka, J. W. I. M. Simons, P. H. M. Lohman, and A. A. van Zeeland.....	1277-1283
Mitotic Recombination Is Responsible for the Loss of Heterozygosity in Cultured Murine Cell Lines. F. Kenneth Nelson, Wayne Frankel, and T. V. Rajan.....	1284-1288
A Plant DNA-Binding Protein That Recognizes 5-Methylcytosine Residues. Daoling Zhang, Kenneth C. Ehrlich, Prakash C. Supakar, and Melanie Ehrlich.....	1351-1356
Acquisition and Processing of a Conditional Dicentric Chromosome in <i>Saccharomyces cerevisiae</i>. Alison Hill and Kerry Bloom.....	1368-1370

Date of Issue: 24 February 1989

p185^{HER2} Monoclonal Antibody Has Antiproliferative Effects In Vitro and Sensitizes Human Breast Tumor Cells to Tumor Necrosis Factor

ROBERT M. HUDZIAK,¹ GAIL D. LEWIS,² MARCY WINGET,³ BRIAN M. FENDLY,³ H. MICHAEL SHEPARD,²
AND AXEL ULLRICH^{1†*}

*Departments of Developmental Biology,¹ Pharmacological Sciences,² and Medicinal and Analytical Chemistry,³
Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080*

Received 3 October 1988/Accepted 8 December 1988

The *HER2/c-erbB-2* gene encodes the epidermal growth factor receptorlike human homolog of the rat *neu* oncogene. Amplification of this gene in primary breast carcinomas has been shown to correlate with poor clinical prognosis for certain cancer patients. We show here that a monoclonal antibody directed against the extracellular domain of p185^{HER2} specifically inhibits the growth of breast tumor-derived cell lines overexpressing the *HER2/c-erbB-2* gene product and prevents *HER2/c-erbB-2*-transformed NIH 3T3 cells from forming colonies in soft agar. Furthermore, resistance to the cytotoxic effect of tumor necrosis factor alpha, which has been shown to be a consequence of *HER2/c-erbB-2* overexpression, is significantly reduced in the presence of this antibody.

HER2/c-erbB-2, the human homolog of the rat proto-oncogene *neu* (4, 34), encodes a 1,255-amino-acid glycoprotein with extensive homology to the human epidermal growth factor (EGF) receptor (4, 21, 33, 34, 42). The *HER2/c-erbB-2* gene product, p185^{HER2}, has all of the structural features and many of the functional properties of subclass I growth factor receptors (reviewed in references 43 and 44), including cell surface location and an intrinsic tyrosine kinase activity. However, the ligand for this putative growth factor receptor has not yet been identified.

Amplification of the *HER2/c-erbB-2* gene has been found in human salivary gland and gastric tumor-derived cell lines (13, 34), as well as in mammary gland carcinomas (21, 22, 40, 42). Slamon et al. (35) surveyed 189 primary breast adenocarcinomas and determined that the *HER2/c-erbB-2* gene was amplified in about 30% of the cases. Most importantly, *HER2/c-erbB-2* amplification was correlated with a negative prognosis and high probability of relapse. Similar although less frequent amplification of the *HER2/c-erbB-2* gene has been reported for gastric and colon adenocarcinomas (45, 46). Experiments with NIH 3T3 cells also suggest a direct role for the overexpressed, structurally unaltered *HER2/c-erbB-2* gene product p185^{HER2} in neoplastic transformation. High levels of *HER2/c-erbB-2* gene expression attained by coamplification of the introduced gene with dihydrofolate reductase by methotrexate selection (18) or by using a strong promoter (6) was shown to transform NIH 3T3 fibroblasts. Only cells with high levels of p185^{HER2} are transformed, i.e., have an altered morphology, are anchorage independent, and will form tumors in athymic mice.

Overexpression of p185^{HER2} may, furthermore, contribute to malignant tumor development by allowing tumor cells to evade one component of the antitumor defenses of the body, the activated macrophage (17). Macrophages play an important role in immune surveillance against neoplastic growth in vivo (1, 2, 38), and Urban et al. (39) have shown that tumor

cells made resistant to macrophages display enhanced tumorigenicity. Tumor necrosis factor alpha (TNF- α) has been shown to play a role in activated macrophage-mediated tumor cell killing in vitro (3, 11, 23, 29, 39). NIH 3T3 cells transformed by a transfected and amplified *HER2/c-erbB-2* cDNA show increased resistance to the cytotoxic effects of activated macrophages or TNF- α in direct correlation with increased levels of p185^{HER2} expression. Furthermore, breast tumor cell lines with high levels of p185^{HER2} exhibit resistance to TNF- α . Resistance to host antitumor defenses could facilitate the escape of cells from a primary tumor to establish metastases at distant sites.

To further investigate the consequences of alteration in *HER2/c-erbB-2* gene expression in mammary gland neoplasia and to facilitate investigation of the normal biological role of the *HER2/c-erbB-2* gene product, we have prepared monoclonal antibodies against the extracellular domain of p185^{HER2}. One monoclonal antibody (4D5) was characterized in more detail and was shown to inhibit in vitro proliferation of human breast tumor cells overexpressing p185^{HER2} and, furthermore, to increase the sensitivity of these cells to the cytotoxic effects of TNF- α .

MATERIALS AND METHODS

Cells and cell culture. Human tumor cell lines were obtained from the American Type Culture Collection. The mouse fibroblast line NIH 3T3/HER2-3₄₀₀, expressing an amplified *HER2/c-erbB-2* cDNA under simian virus 40 early promoter control, and the vector-transfected control cell line NIH 3T3/CVN have been described previously (18).

Cells were cultured in a 1:1 mixture of Dulbecco modified Eagle medium and Ham nutrient mixture F-12 supplemented with 2 mM glutamine, 100 u of penicillin per ml, 100 μ g of streptomycin per ml, and 10% serum. Human tumor cell lines were cultured with fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.); NIH 3T3 derivatives were cultured with calf serum (Hyclone Laboratories, Inc., Logan, Utah.).

Immunization. Female BALB/c mice were immunized with NIH 3T3/HER2-3₄₀₀ cells expressing high levels of

* Corresponding author.

† Present address: Max-Planck-Institut für Biochemie, 8033 Martinsried, Federal Republic of Germany.

p185^{HER2}. The cells were washed once with phosphate-buffered saline (PBS) and detached from the plate with PBS containing 25 mM EDTA. After low-speed centrifugation, the cells were suspended in cold PBS (2×10^7 cells per ml). Each mouse was injected intraperitoneally with 0.5 ml of this cell suspension on weeks 0, 2, 5, and 7.

On weeks 9 and 13, 100 μ l of a Triton X-100 membrane preparation of p185^{HER2}, partially purified by wheat germ agglutinin chromatography (700 μ g of protein per ml) (25), was administered intraperitoneally. Three days before fusion, 100 μ l of the enriched p185^{HER2} protein was administered intravenously.

Fusion and screening. Mice with high antibody titers as determined by immunoprecipitation of p185^{HER2} were sacrificed, and their splenocytes were fused as described previously (26). Spleen cells were mixed at a 4:1 ratio with the fusion partner, mouse myeloma cell line X63-Ag8.653 (20), in the presence of 50% polyethylene glycol 4000. Fused cells were plated at a density of 2×10^5 cells per well in 96-well microdilution plates. The hypoxanthine-azaserine (12) selection for hybridomas was begun 24 h later. Beginning at day 10 postfusion, supernatants from hybridoma-containing wells were tested for the presence of antibodies specific for p185^{HER2} by an enzyme-linked immunosorbent assay with the wheat germ agglutinin chromatography-purified p185^{HER2} preparation (28). Enzyme-linked immunosorbent assay-positive supernatants were confirmed by immunoprecipitation and cloned twice by limiting dilution.

Large quantities of specific monoclonal antibodies were produced by preparation of ascites fluid; antibodies were then purified on protein A-Sepharose columns (Fermentech, Inc., Edinburgh, Scotland) and stored sterile in PBS at 4°C.

Immunoprecipitations and antibodies. Cells were harvested by trypsinization, counted in a Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.), and plated 24 h before being harvested for analysis of p185^{HER2} expression. Cells were lysed at 4°C with 0.8 ml of HNEG lysis buffer (18) per 100-mm plate. After 10 min, 1.6 ml of lysis dilution buffer (HNEG buffer with 1% bovine serum albumin and 0.1% Triton X-100) was added to each plate, and the extracts were clarified by centrifugation at $12,000 \times g$ for 5 min.

Antibodies were added to the cell extracts and allowed to bind at 4°C for 2 to 4 h. Immune complexes were collected by adsorption to protein A-Sepharose beads for 20 min and washed three times with 1 ml of HNEG buffer-0.1% Triton X-100. Autophosphorylation reactions were carried out for 20 min at 4°C in 50 μ l of HNEG wash buffer containing 5 mM MnCl₂ and 3 μ Ci of [γ -³²P]ATP (5,000 Ci/mmol, Amersham Corp., Arlington Heights, Ill.). The autophosphorylation reaction conditions have been described previously (18). Proteins were separated on sodium dodecyl sulfate (SDS)-7.5% polyacrylamide gels and analyzed by autoradiography.

The polyclonal antibody, G-H2CT17, recognizing the carboxy-terminal 17 amino acids of p185^{HER2}, has been described previously (18). The anti-EGF receptor monoclonal antibody 108 (16) was provided by Joseph Schlessinger, Rorer Biotechnology, Inc.

Fluorescence-activated cell sorting. SK-BR-3 human breast tumor cells overexpressing the *HER2/c-erbB-2* gene (17, 22) or A431 human squamous carcinoma cells overexpressing the EGF receptor gene (14) were grown in T175 flasks. They were detached from the flasks by treatment with 25 mM EDTA-0.15 M NaCl, collected by low-speed centrifugation, and suspended at 1×10^6 cells per ml in PBS-1% fetal bovine serum. One milliliter of each cell line was incubated with 10 μ g of either anti-*HER2/c-erbB-2* monoclonal antibody (4D5)

or a control antibody (40.1.H1) recognizing the hepatitis B surface antigen. The cells were washed twice and suspended on ice for 30 min in 1 ml of PBS-1% fetal bovine serum containing 10 μ g of goat anti-mouse immunoglobulin G F(ab')₂ fragments conjugated with fluorescein isothiocyanate dye (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Unbound fluorescein dye was removed by two further washes. The cells were suspended at 2×10^6 per ml in PBS-1% fetal bovine serum and analyzed with an EPICS 753 (Coulter) fluorescence-activated cell sorter. Fluorescein was excited by 300 mW of 488-nm argon laser light, and the emitted light was collected with a 525-nm band-pass filter with a 10-nm band width.

Down-regulation assay. SK-BR-3 cells were plated at 1.5×10^5 cells per 35-mm culture dish in normal medium. After a 6-h period to allow attachment, the medium was replaced by 1.5 ml of methionine-free labeling medium containing 150 μ Ci of [³⁵S]methionine per ml and 2% dialyzed fetal bovine serum. The cells were metabolically labeled for 14 h and then chased with medium containing 2% dialyzed serum and unlabeled methionine. Either a control monoclonal antibody (40.1.H1) or anti-p185^{HER2} (4D5) was added to a final concentration of 2.5 μ g/ml. At 0, 5, and 11 h, extracts were prepared with 0.3 ml of lysis solution and 0.6 ml of dilution buffer. The p185^{HER2} was immunoprecipitated with 2.5 μ l of polyclonal antibody G-H2CT17. The washed immune complexes were dissolved in sample buffer, electrophoresed on a SDS-7.5% polyacrylamide gel, and analyzed by autoradiography. Each time point determination was performed in duplicate. Autoradiograph band intensities were quantitated by using a scanner (Ambis Systems).

Cell proliferation assays. The anti-p185^{HER2} monoclonal antibodies were characterized by using the breast tumor cell line SK-BR-3. Cells were detached by using 0.25% (vol/vol) trypsin and suspended in complete medium at a density of 4

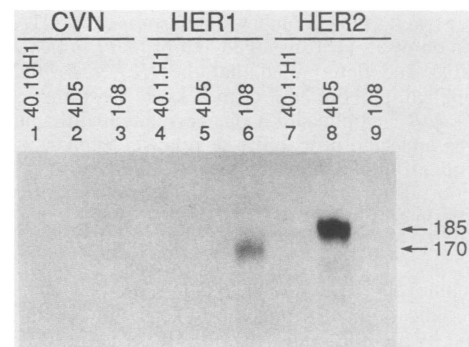


FIG. 1. Specificity of monoclonal antibody 4D5. Three cell lines, NIH 3T3/CVN, NIH 3T3/HER1-EGF receptor, and NIH 3T3/HER2-3₄₀₀, were plated out at 2.0×10^6 in 100-mm culture dishes. At 24 h, Triton X-100 lysates were prepared and divided into three portions. Either an irrelevant monoclonal antibody (6 μ g of anti-hepatitis B virus surface antigen, 40.1.H1; lanes 1, 4, and 7), anti-p185^{HER2} monoclonal antibody 4D5 (6 μ g; lanes 2, 5, and 8), or anti-EGF receptor monoclonal antibody 108 (6 μ g; lanes 3, 6, and 9) was added and allowed to bind at 4°C for 4 h. The immune complexes were collected with 30 μ l of protein A-Sepharose. Rabbit anti-mouse immunoglobulin (7 μ g) was added to each 4D5 immunoprecipitation to improve the binding of this monoclonal antibody to the protein A-coated beads. Proteins were labeled by autophosphorylation and separated on an SDS-7.5% polyacrylamide gel. The gel was exposed to film at -70°C for 4 h with an intensifying screen. The arrows show the positions of proteins of *M*, 185,000 and 170,000.

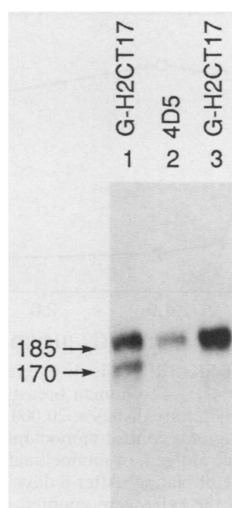


FIG. 2. Binding of monoclonal antibody 4D5 to unglycosylated receptor. NIH 3T3/HER2-3₄₀₀ cells were plated into two 100-mm plates at 2×10^6 cells per plate. After 14 h, the antibiotic tunicamycin was added to one plate at 3 $\mu\text{g}/\text{ml}$. After a further 5.5 h of incubation, Triton X-100 lysates were then prepared from each plate. Immunoprecipitations, the autophosphorylation reaction, and SDS-polyacrylamide gel electrophoresis were performed as described in the legend to Fig. 1. Lanes: 1, tunicamycin-treated cell lysate (one-third of a plate) immunoprecipitated with 2.5 μl of a polyclonal antibody directed against the C terminus of p185^{HER2}; 2, tunicamycin-treated cell lysate (one-third of a plate) immunoprecipitated with 6 μg of 4D5; 3, untreated control lysate (one-third of a plate) immunoprecipitated with the polyclonal antibody. The arrows show the locations of proteins of M_r 185,000 and 170,000.

$\times 10^5$ cells per ml. Aliquots of 100 μl (4×10^4 cells) were plated into 96-well microdilution plates, the cells were allowed to adhere, and 100 μl of media alone or media containing monoclonal antibody (final concentration, 5 $\mu\text{g}/\text{ml}$) was then added. After 72 h, plates were washed twice with PBS (pH 7.5), stained with crystal violet (0.5% in methanol), and analyzed for relative cell proliferation as described previously (36).

For assays in which monoclonal antibodies were combined with recombinant human TNF- α (5.0×10^7 U/mg; Genentech, Inc.), cells were plated and allowed to adhere as described above. Following cell adherence, control medium alone or medium containing monoclonal antibodies was added to a final concentration of 5 $\mu\text{g}/\text{ml}$. Cultures were incubated for another 4 h, and then increasing concentrations of TNF- α were added to a final volume of 200 μl . Following 72 h of incubation, the relative cell number was determined by crystal violet staining. Some samples were analyzed by crystal violet staining following cell adherence for determination of the initial cell number.

RESULTS

Specificity of monoclonal antibody 4D5. Monoclonal antibodies directed against the extracellular domain of p185^{HER2} were prepared by immunizing mice with NIH 3T3 cells transfected with a *HER2/c-erbB-2* cDNA (HER2-3₄₀₀) (17, 18) and overexpressing the corresponding gene product, p185^{HER2}. One antibody exhibited several interesting biological properties and was chosen for further characterization. Antibody 4D5 specifically immunoprecipitated a single ³²P-

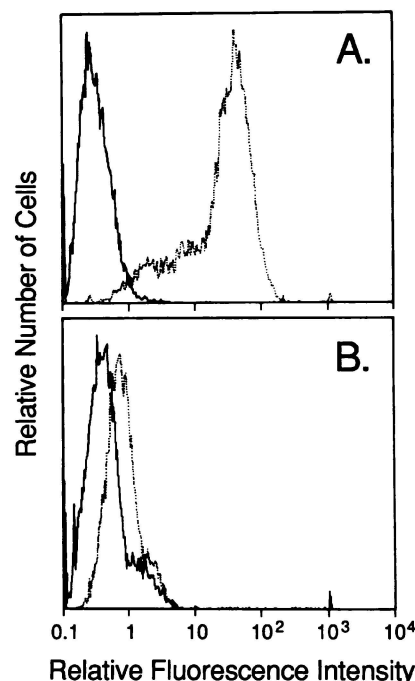


FIG. 3. Fluorescence-activated cell sorter histograms of human tumor cells binding anti-p185 monoclonal antibody 4D5. —, Binding by the control antibody, 40.1.H1, directed against the hepatitis B surface antigen; ·····, binding by the anti-*HER2/c-erbB-2* antibody, 4D5. The antibodies were first allowed to react with the cell surface. After a wash step, bound antibody was labeled by addition of fluorescein-conjugated F(ab')₂ fragment of goat anti-mouse immunoglobulin G. (A) Binding of the antibodies to the human breast tumor line SK-BR-3, which contains an amplification of the *HER2/c-erbB-2* gene and expresses high levels of the *HER2/c-erbB-2* gene product p185^{HER2}. (B) Binding of the same antibodies to the human squamous epithelial cell line A431. This cell line expresses low levels of mRNA for *HER2/c-erbB-2* and high levels (2×10^6 receptors per cell) of the EGF receptor.

labeled protein of M_r 185,000 from NIH 3T3 cells expressing p185^{HER2} (Fig. 1, lane 8). This antibody did not cross-react with the human EGF receptor (HER1; Fig. 1, lane 5), even when overexpressed in a mouse NIH 3T3 background (Fig. 1, lane 6). Furthermore, it did not immunoprecipitate any proteins from NIH 3T3 cells transfected with a control plasmid (pCVN) which expresses the neomycin resistance and dihydrofolate reductase genes only (Fig. 1, lane 2).

To determine the nature of the epitope recognized by 4D5, NIH 3T3/HER2-3₄₀₀ cells were treated with tunicamycin, which prevents addition of N-linked oligosaccharides to proteins (15, 41). Cells treated with this antibiotic for 5.5 h contained two proteins which were immunoprecipitated by a polyclonal antibody against the carboxy-terminal peptide of p185^{HER2} (Fig. 2, lane 1). The polypeptide of 170,000 M_r represents unglycosylated p185^{HER2}. The upper band of ca. 185,000 M_r comigrated with glycosylated p185^{HER2} from untreated cells (Fig. 2, lane 3). Monoclonal antibody 4D5 efficiently immunoprecipitated only the glycosylated form of p185^{HER2} (Fig. 2, lane 2). This experiment suggests either that the epitope recognized by 4D5 consists partly of carbohydrate, or, alternatively, that the antibody recognizes a conformation of the protein achieved only when it is glycosylated.

TABLE 1. Inhibition of SK-BR-3 proliferation by anti-p185^{HER2} monoclonal antibodies^a

Monoclonal antibody	Relative cell proliferation ^b
7C2	79.3 ± 2.2
2C4	79.5 ± 4.4
7D3	83.8 ± 5.9
4D5	44.2 ± 4.4
3E8	66.2 ± 2.4
6E9	98.9 ± 3.6
7F3	62.1 ± 1.4
3H4	66.5 ± 3.9
2H11	92.9 ± 4.8
40.1.H1	105.8 ± 3.8
4F4	94.7 ± 2.8

^a SK-BR-3 breast tumor cells were plated as described in Materials and Methods. Following adherence, medium containing 5 µg of either anti-p185^{HER2} or control monoclonal antibodies (40.1.H1 and 4F4) per ml were added.

^b Relative cell proliferation was determined by crystal violet staining of the monolayers after 72 h. Values are expressed as a percentage of results with untreated control cultures (100%).

The binding of monoclonal antibody 4D5 to human tumor cell lines was investigated by fluorescence-activated cell sorting (Fig. 3). This antibody was bound to the surface of cells expressing p185^{HER2}. Figure 3A shows the 160-fold increase in cellular fluorescence observed when 4D5 was added to SK-BR-3 breast adenocarcinoma cells relative to a control monoclonal antibody. This cell line contains an amplified *HER2/c-erbB-2* gene and expresses high levels of p185^{HER2} (17, 22). In contrast, the squamous carcinoma cell line A431, which expresses about 2×10^6 EGF receptors per cell (14) but only low levels of p185^{HER2} (4), exhibited only a twofold increase in fluorescence with 4D5 (Fig. 3B) when compared with a control monoclonal antibody.

The binding of 4D5 correlated with the levels of p185^{HER2} expressed by these two cell lines. SK-BR-3 cells, expressing high levels of p185^{HER2}, showed an 80-fold increase in relative fluorescence intensity compared with A431 cells. This experiment demonstrates that 4D5 specifically recognizes the extracellular domain of p185^{HER2}.

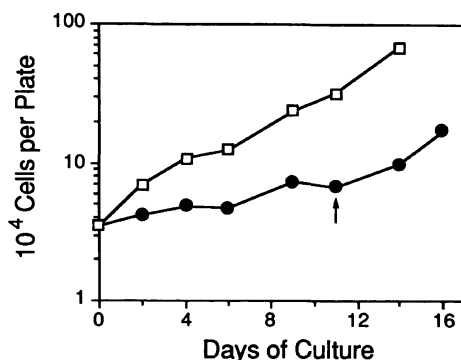


FIG. 4. Growth curve of SK-BR-3 cells treated with anti-*HER2/c-erbB-2* monoclonal antibody 4D5. Cells were plated into 35-mm culture dishes at 20,000 cells per plate in medium containing 2.5 µg of either control antibody (40.1.H1, anti-hepatitis B surface antigen) (□) or anti-p185^{HER2} antibody 4D5 (●) per ml. On the indicated days, cells were trypsinized and counted in a Coulter counter. The determination for each time point and each antibody was done in duplicate, and the counts were averaged. The arrow indicates the day the cells were refed with medium without antibodies.

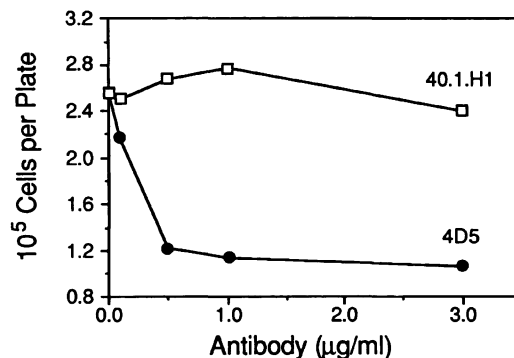


FIG. 5. Growth of SK-BR-3 cells in different concentrations of monoclonal antibody 4D5. The human breast tumor line SK-BR-3 was plated into 35-mm culture dishes at 20,000 cells per dish. Either 0.1, 0.5, 1.0, or 3.0 µg of a control monoclonal antibody (40.1.H1, anti-hepatitis B surface antigen) or monoclonal 4D5 antibody per ml was added at the time of plating. After 8 days of growth, the plates were trypsinized and the cells were counted in a Coulter counter. Each concentration of antibody was plated and counted in duplicate, and the cell numbers were averaged.

Effects on cell proliferation. We used the human mammary gland adenocarcinoma cell line, SK-BR-3, to determine whether monoclonal antibodies directed against the extracellular domain of p185^{HER2} had any effect on the proliferation of cell lines overexpressing this receptorlike protein. SK-BR-3 cells were coincubated with several *HER2/c-erbB-2*-specific monoclonal antibodies or with either of two different control monoclonal antibodies (40.1.H1, directed against the hepatitis B surface antigen; 4F4, directed against recombinant human gamma interferon). Most anti-*HER2/c-erbB-2* monoclonal antibodies which recognize the extracellular domain inhibited the growth of SK-BR-3 cells (Table

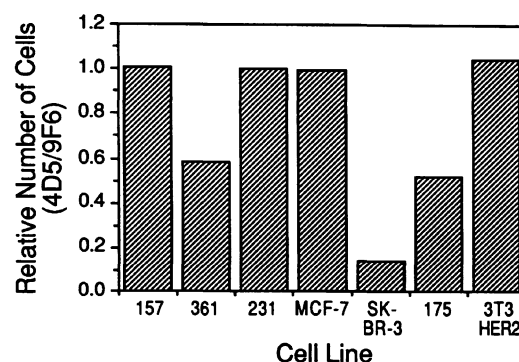


FIG. 6. Screening of breast tumor cell lines for growth inhibition by monoclonal antibody 4D5. Each cell line was plated in 35-mm culture dishes at 20,000 cells per dish. Either a control monoclonal antibody (9F6, anti-human immunodeficiency virus gp120) or the anti-p185^{HER2} monoclonal antibody 4D5 was added on day 0 to 2.5 µg/ml. Because the different cell lines grow at different rates, the cell lines NIH 3T3/HER2-3₄₀₀ and SK-BR-3 were counted after 6 days, cell lines MDA-MB-157, MDA-MB-231, and MCF-7 were counted after 9 days, and cell lines MDA-MB-175VII and MDA-MB-361 were counted after 14 days. The difference in growth between cells treated with 4D5 and 40.1.H1 is expressed as the ratio of cell numbers with 4D5 versus a control monoclonal antibody, 9F6. Each cell line was assayed in duplicate for each antibody, and the counts were averaged.

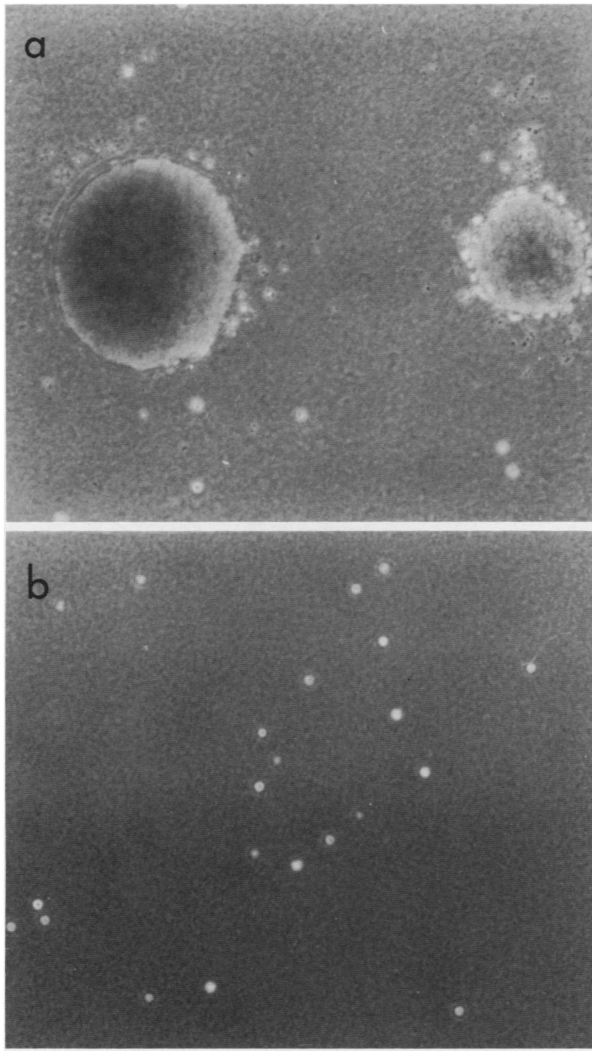


FIG. 7. Inhibition of anchorage-independent growth of NIH 3T3/HER2-3₄₀₀ cells by 4D5. Cells (20,000 per 60-mm plate) were plated in 0.2% soft agar over a 0.4% agar base. After 3 weeks, the plates were photographed at $\times 100$ magnification by using a Nikon microscope with phase-contrast optics. (a) HER2-3₄₀₀ cells plated in agar containing 200 ng of a control antibody (TF-C8) per ml. (b) The same cells plated in agar containing 200 ng of 4D5 per ml.

1). Maximum inhibition was obtained with monoclonal antibody 4D5, which inhibited cellular proliferation by 56%. The control antibodies had no significant effect on cell growth.

Figure 4 compares the growth of SK-BR-3 cells in the presence of either a control antibody, 40.1.H1, or the anti-p185^{HER2} antibody. Proliferation of the cells was inhibited when antibody 4D5 was present. The generation time increased from 3.2 to 12.2 days. To determine whether 4D5 treatment was cytostatic or cytotoxic, antibody was removed by medium change 11 days after treatment. The cells resumed growth at a nearly normal rate, suggesting that the antibody affected cell growth rather than cell viability. The dose-response curve (Fig. 5) showed that a concentration of 200 ng/ml inhibited growth by 50%, whereas maximum

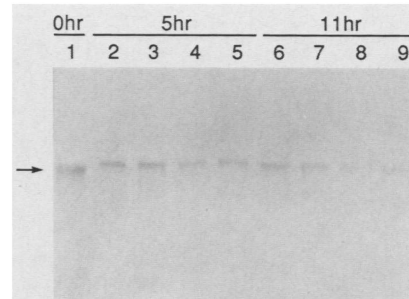


FIG. 8. Effect of antibody binding on p185^{HER2} turnover. SK-BR-3 cells were labeled for 14 h with [³⁵S]methionine. The label was then chased with cold methionine and either an irrelevant monoclonal antibody (40.1.H1, anti-hepatitis B surface antigen) or 4D5 was added to 2.5 μ g/ml. The cells on the plates were lysed at 0, 5, and 11 h, and ³⁵S-labeled p185^{HER2} was quantitated by immunoprecipitation with the C-terminal specific polyclonal antibody. The 5- and 11-h time point determinations were performed in duplicate for each of the two antibodies. Proteins were separated by SDS-polyacrylamide gel electrophoresis. The fluor-treated gel was exposed to film for 4 h at room temperature. The arrow indicates the position of a protein of M_r 185,000. Band intensities were quantitated by using an Ambis Systems scanner. Lanes; 1, 0 h; lanes 2 and 3, 40.1.H1 (5 h); lanes 4 and 5, 4D5 (5 h); lanes 6 and 7, 40.1.H1 (11 h); lanes 8 and 9, 4D5 (11 h).

effects were achieved by using a concentration of between 0.5 and 1 μ g/ml.

The effect of 4D5 on the proliferation of six additional breast tumor cell lines, as well as mouse NIH 3T3 fibroblasts transformed by p185^{HER2} overexpression (NIH 3T3/HER2-3₄₀₀), was tested in monolayer growth assays. Cells were plated at low density in medium containing 2.5 μ g of either a control antibody or 4D5 per ml. When the cultures approached confluency, cells were removed with trypsin and counted. 4D5 did not have any significant effect on the growth of the MCF-7, MDA-MB-157, MDA-MB-231, or NIH 3T3/HER2-3₄₀₀ cell lines (Fig. 6). It did, however, significantly affect the growth of the cell lines MDA-MB-361 (58% of control) and MDA-MB-175-VII (52% of control), which express high levels of p185^{HER2} (17).

Interestingly, monoclonal antibody 4D5 had no effect on the monolayer growth of the NIH 3T3/HER2-3₄₀₀ cell line. However, it completely prevented colony formation by these cells in soft agar (Fig. 7), a property which had been induced by *HER2/c-erbB-2* amplification (18). In the presence of 200 ng of a control monoclonal antibody (antitissue factor, TC-C8) per ml, 116 (average of two plates) soft-agar colonies were counted, while the same cells plated simultaneously into soft agar containing 200 ng of 4D5 per ml did not yield any colonies.

Monoclonal antibody 4D5 down-regulates p185^{HER2}. To determine whether the antiproliferative effect of 4D5 was due to enhanced degradation of p185^{HER2}, we measured its rate of turnover in the presence or absence of antibody. p185^{HER2} was metabolically labeled by culturing SK-BR-3 cells for 14 h in the presence of [³⁵S]methionine. Cells were then chased for various times, and either a control antibody or 4D5 was added at the beginning of the chase period. At 0, 5, and 11 h, cells were lysed and p185^{HER2} levels were assayed by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. p185^{HER2} is degraded more rapidly after exposure of SK-BR-3 cells to 4D5 (Fig. 8). Densitometric evaluation of the data showed that the p185^{HER2} half-life of

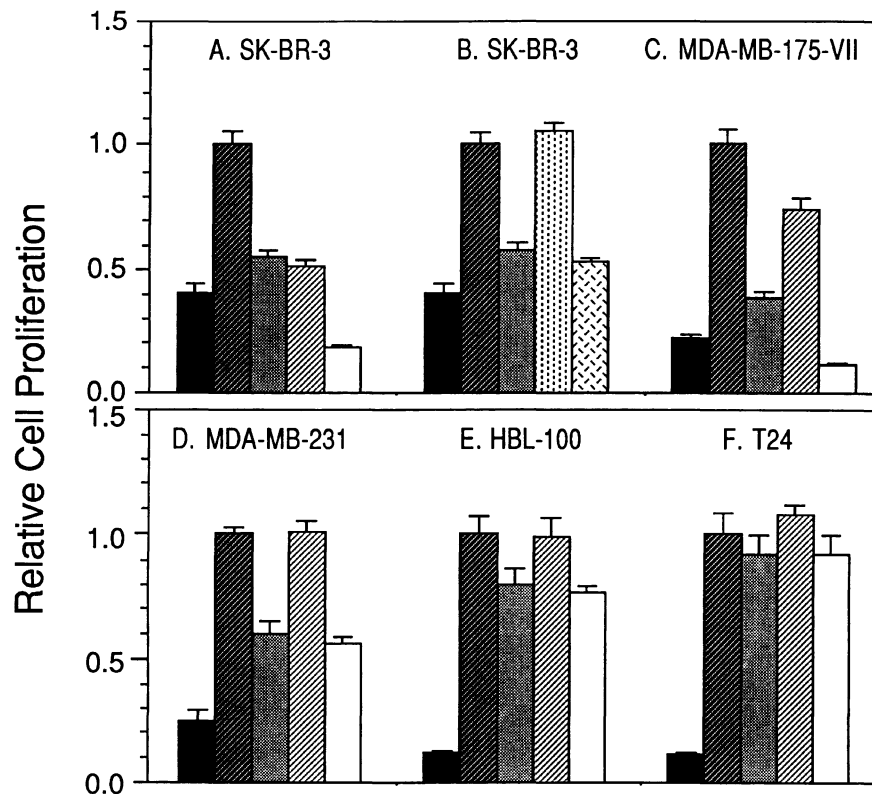


FIG. 9. Monoclonal antibody 4D5 sensitizes breast tumor cells to the cytotoxic effects of TNF- α . Cells were plated in 96-well microdilution plates (4×10^4 cells per well for SK-BR-3, MDA-MB-175-VII, and MDA-MB-231; 10^4 cells per well for HBL-100 and T24) and allowed to adhere for 2 h. Anti-*HER2/c-erbB-2* monoclonal antibody 4D5 (5 μ g/ml) or anti-hepatitis B surface antigen monoclonal antibody 40.1.H1 (5 μ g/ml) was then added for a 4-h incubation prior to the addition of TNF- α to a final concentration of 10^4 units/ml. After 72 h, the monolayers were washed twice with PBS and stained with crystal violet dye for determination of relative cell proliferation. In addition, some cell monolayers were stained with crystal violet following adherence in order to determine the initial cell density for comparison with cell densities measured after 72 h. The symbols denote initial cell density (■), untreated (control) cells (□), cells treated with TNF- α (■), 4D5 (▨), TNF- α plus 4D5 (▧), 40.1.H1 (▤); or TNF- α plus 40.1.H1 (▦).

7 h decreased to 5 h in the presence of antibody (data not shown).

Monoclonal antibody 4D5 enhances TNF- α cytotoxicity. The addition of certain growth factors to tumor cells has been shown to increase their resistance to the cytotoxic effects of TNF- α (37). A prediction based on these findings would be that expression of oncogenes that mimic or replace growth factor receptor function may also increase the resistance of cells to this cytokine. Recently, it was shown that overexpression of the putative growth factor receptor p185^{HER2} in NIH 3T3 cells caused an increase in the resistance of these cells to TNF- α (17). Furthermore, breast tumor cell lines with high levels of p185^{HER2} also exhibited TNF- α resistance.

To further investigate the mechanism by which the 4D5 antibody inhibited cell growth, we investigated the response of three breast tumor cell lines to TNF- α in the presence or absence of this antibody. If the anti-p185^{HER2} monoclonal antibody 4D5 inhibited proliferation of breast tumor cells by interfering with the signalling functions of p185^{HER2}, addition of this antibody would be expected to enhance the sensitivity of tumor cells to TNF- α . Both SK-BR-3 (Fig. 9A) and MDA-MB-175-VII (Fig. 9C) were growth inhibited by both the monoclonal antibody 4D5 (5 μ g/ml; 50% and 25% inhibition, respectively) and high concentrations of TNF- α

(1×10^4 units/ml; 50% and 60% inhibition, respectively). However, the combination of TNF- α and monoclonal antibody 4D5 reduced the SK-BR-3 and MDA-MB-175-VII tumor cell number to a level below that initially plated, indicating the induction of a cytotoxic response. In a separate experiment, SK-BR-3 cell viability was determined directly by using trypan blue dye exclusion, yielding identical results to those described above that were obtained by using crystal violet staining (data not shown). A control monoclonal antibody, 40.1.H1, did not inhibit SK-BR-3 breast tumor cell proliferation, nor did it induce an enhanced sensitivity of this cell line to the cytotoxic effects of TNF- α (Fig. 9B). In addition, the growth of the breast tumor cell line MDA-MB-231, which does not express detectable levels of p185^{HER2} (17), was unaffected by monoclonal antibody 4D5, and the growth inhibition seen with the combination of 4D5 and TNF- α was similar to that observed with TNF- α alone (Fig. 9D). Furthermore, neither HBL-100 (30), a nontransformed but immortalized human breast epithelial cell line (Fig. 9E), nor T24 (27), a human bladder carcinoma cell line (Fig. 9F), expressed high levels of p185^{HER2} (data not shown), and neither demonstrated growth inhibition by 4D5 or an enhanced growth-inhibitory or cytotoxic response to the combination of TNF- α and monoclonal antibody 4D5. These results demonstrate that only tumor cells which

overexpress p185^{HER2} will become sensitized to the cytotoxic effects of TNF- α by antibody 4D5.

DISCUSSION

We have prepared monoclonal antibodies against the extracellular domain of the *HER2/c-erbB-2* gene product, p185^{HER2}, and have found that one of these, 4D5, strongly inhibits the growth of several breast tumor cell lines and furthermore sensitizes p185^{HER2}-overexpressing breast carcinoma cell lines SK-BR-3 and MDA-MB-175-VII to the cytotoxic effects of TNF- α . Monoclonal antibody 4D5 is specific for p185^{HER2} and shows no cross-reactivity with the closely related human EGF receptor expressed in mouse fibroblasts. Of six mammary carcinoma cell lines tested, only the three lines which express high levels of p185^{HER2} (SK-BR3, MBA-MB-175, and MDA-MD-361 [17]) were growth inhibited, and 4D5 did not inhibit the proliferation of a nontransformed human breast epithelial cell line, HBL-100, or the bladder carcinoma cell line T24.

In the presence of the antibody, the inhibition of SK-BR-3 cell growth was nearly complete, but the effect was cytostatic rather than cytotoxic. This property of 4D5 is similar to that described for a subset of monoclonal antibodies to the EGF receptor (19, 31, 32) which inhibit the growth of A431 cells, a human squamous epithelial carcinoma line expressing high levels of the EGF receptor. In this case, these inhibitory antibodies compete with radiolabeled EGF for binding to the receptor, and antibodies which do not block EGF binding have no effect on A431 cell growth. It has been suggested (J. Mendelsohn and H. Masui, Clin. Res. 35:600A, 1987) that these antibodies inhibit cell growth by interfering with an autocrine system involving the EGF receptor and an essential growth factor, transforming growth factor alpha, that is produced by the cells (5). It is therefore intriguing to speculate that antibody 4D5 analogously interferes with ligand binding to the *HER2/c-erbB-2* gene product. Since an appropriate ligand for the putative *HER2/c-erbB-2* receptor has not yet been identified, this possibility cannot yet be tested directly.

The 4D5 antibody had no effect on the growth of NIH 3T3 cells transformed by *HER2/c-erbB-2* overexpression. However, it reversed one property conferred on these cells by amplification of the *HER2/c-erbB-2* cDNA: the formation of colonies in soft agar was prevented by 200 ng of 4D5 antibody per ml. This result is similar to those obtained by Drebin et al. (8) with a monoclonal antibody to the rat *neu* oncogene-encoded p185^{neu}. They also observed that an anti-p185^{neu} monoclonal antibody inhibited colony growth in soft agar and tumor formation by *neu*-transformed NIH 3T3 cells in athymic mice (7-10). This effect was attributed to a lowering p185^{neu} levels by an increase in receptor turnover triggered by antibody binding. The apparent discrepancy between 4D5 effects on proliferation of breast tumor cells versus transfected mouse fibroblast cells is most probably a reflection of the fact that SK-BR-3 cells are authentic cancer cells, in contrast to the NIH 3T3 model system. Whereas SK-BR-3 cells may have evolved to be dependent on *HER2/c-erbB-2*-mediated signals for both growth and transformation characteristics, NIH 3T3 cells have acquired a transformed phenotype only as a result of *HER2/c-erbB-2* overexpression, but may proliferate normally in response to other serum growth factors, even in the presence of blocking anti-p185^{HER2} antibody.

Previous work has shown that high-level expression of p185^{HER2} will transform NIH 3T3 cells and has suggested a casual role for amplification of the *HER2/c-erbB-2* gene in

mammary gland neoplasia. We have shown here that *HER2/c-erbB-2* gene overexpression in NIH 3T3 cells is associated with increased resistance to the monokine TNF- α and that breast tumor cell lines which overexpress p185^{HER2} are resistant to the cytotoxic effects of TNF- α . The mechanism by which 4D5 inhibits breast tumor cell proliferation and reverses phenotypes associated with high levels of p185^{HER2} expression, such as resistance to TNF- α , is not clear. However, these results suggest that in addition to its ability to transform cells by virtue of overexpression (6, 18), *HER2/c-erbB-2* could play a role in tumor progression by allowing tumor cells overexpressing p185^{HER2} to evade one component of the antitumor immunosurveillance of the host, the activated macrophage (17). These properties of the *HER2/c-erbB-2* gene product may in part explain the aggressive, single-step induction of mammary adenocarcinoma in transgenic mice bearing the *neu* oncogene (24), which encodes the mutated rat homolog of p185^{HER2}.

The experiments presented here demonstrate that a monoclonal antibody which recognizes the extracellular domain of p185^{HER2} inhibits the proliferation of breast tumor cells which overexpress this receptorlike protein. Moreover, treatment with this antibody also sensitizes these tumor cells to the cytotoxic effects of TNF- α . Monoclonal antibodies specific for p185^{HER2} may therefore be useful therapeutic agents for the treatment of human neoplasias, including certain mammary carcinomas, which are characterized by the overexpressing of p185^{HER2}.

ACKNOWLEDGMENTS

We thank Bill Lagrimas for help with the immunization procedure and Mary Napier and Michael Lipari for providing the wheat germ purified p185^{HER2} preparation. We are grateful to Jeanne Arch for her patience and skill in typing this manuscript.

LITERATURE CITED

1. Adams, D. O., and C. F. Nathan. 1983. Molecular mechanisms in tumor cell killing by activated macrophages. *Immunol. Today* 4:166-170.
2. Adams, D. O., and R. Snyderman. 1978. Do macrophages destroy nascent tumors? *JNCI* 62:1341-1345.
3. Beutler, B., and A. Cerami. 1986. Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature (London)* 320:584-588.
4. Coussens, L., T. L. Yang-Feng, Y.-C. Liao, E. Chen, A. Gray, J. McGrath, P. H. Seeburg, T. W. Libermann, J. Schlessinger, U. Francke, A. Levinson, and A. Ullrich. 1985. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with *neu* oncogene. *Science* 230:1132-1139.
5. Derynck, R., D. V. Goeddel, A. Ullrich, J. U. Gutterman, R. D. Williams, T. S. Bringman, and W. H. Berger. 1987. Synthesis of messenger RNAs for transforming growth factors α and β and the epidermal growth factor receptor by human tumors. *Cancer Res.* 47:707-712.
6. Di Force, P. P., J. H. Pierce, M. H. Kraus, O. Segatto, C. R. King, and S. A. Aaronson. 1987. *erbB-2* is a potent oncogene when overexpressed in NIH/3T3 cells. *Science* 237:178-182.
7. Drebin, J. A., V. C. Link, and M. I. Greene. 1988. Monoclonal antibodies reactive with distinct domains of the *neu* oncogene-encoded p185 molecule exert synergistic anti-tumor effects *in vivo*. *Oncogene* 2:273-277.
8. Drebin, J. A., V. C. Link, and M. I. Greene. 1988. Monoclonal antibodies specific for the *neu* oncogene product directly mediate anti-tumor effects *in vivo*. *Oncogene* 2:387-394.
9. Drebin, J. A., V. C. Link, D. F. Stern, R. A. Weinberg, and M. I. Greene. 1985. Down-modulation of an oncogene protein product and reversion of the transformed phenotype by monoclonal antibodies. *Cell* 41:695-706.
10. Drebin, J. A., V. C. Link, R. A. Weinberg, and M. I. Greene.

1986. Inhibition of tumor growth by a monoclonal antibody reactive with an oncogene-encoded tumor antigen. *Proc. Natl. Acad. Sci. USA* **83**:9129-9133.
11. **Feinman, R., D. Henriksen-deStefano, M. Tsujimoto, and J. Vilcek.** 1987. Tumor necrosis factor is an important mediator of tumor cell killing by human monocytes. *J. Immunol.* **138**: 635-640.
 12. **Foung, S. K. H., D. T. Sasaki, F. C. Grumet, and E. G. Engleman.** 1982. Production of functional human T-T hybridomas in selection of medium lacking aminopterin and thymidine. *Proc. Natl. Acad. Sci. USA* **79**:7484-7488.
 13. **Fukushige, S.-I., K.-I. Matsubara, M. Yoshida, M. Sasaki, T. Suzuki, K. Semba, K. Toyoshima, and T. Yamamoto.** 1986. Localization of a novel *v-erbB*-related gene, *c-erbB-2*, on human chromosome 17 and its amplification in a gastric cancer cell line. *Mol. Cell. Biol.* **6**:955-958.
 14. **Haigler, H., J. F. Ash, S. J. Singer, and S. Cohen.** 1978. Visualization by fluorescence of the binding and internalization of epidermal growth factor in human carcinoma cells. *Proc. Natl. Acad. Sci. USA* **75**:3317-3321.
 15. **Heifetz, A., R. W. Keenan, and A. D. Elbein.** 1979. Mechanism of action of tunicamycin on the UDP-GlcNAc:dolichyl-phosphate GlcNAc-1-phosphate transferase. *Biochemistry* **18**:2186-2191.
 16. **Honegger, A. M., T. J. Dull, S. Felder, E. Van Obberghen, F. Bellot, D. Szapary, A. Schmidt, A. Ullrich, and J. Schlessinger.** 1987. Point mutation at the ATP binding site of EGF receptor abolishes protein-tyrosine kinase activity and alters cellular routing. *Cell* **51**:199-209.
 17. **Hudziak, R. M., G. D. Lewis, M. R. Shalaby, T. E. Eessalu, B. B. Aggarwal, A. Ullrich, and H. M. Shepard.** 1988. Amplified expression of the HER2/ERBB2 oncogene induces resistance to tumor necrosis factor α in NIH 3T3 cells. *Proc. Natl. Acad. Sci. USA* **85**:5102-5106.
 18. **Hudziak, R. M., J. Schlessinger, and A. Ullrich.** 1987. Increased expression of the putative growth factor receptor p185^{HER2} causes transformation and tumorigenesis of NIH 3T3 cells. *Proc. Natl. Acad. Sci. USA* **84**:7159-7163.
 19. **Kawamoto, T., J. D. Sato, A. Le, J. Polikoff, G. H. Sato, and J. Mendelsohn.** 1983. Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an antireceptor monoclonal antibody. *Proc. Natl. Acad. Sci. USA* **80**:1337-1341.
 20. **Kearney, J. F., A. Radbruch, B. Liesegang, and K. Rajewsky.** 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. *J. Immunol.* **123**:1548-1550.
 21. **King, C. R., M. H. Kraus, and S. A. Aaronson.** 1985. Amplification of a novel *v-erbB*-related gene in a human mammary carcinoma. *Science* **229**:974-976.
 22. **Kraus, M. H., N. C. Popescu, S. C. Amsbaugh, C. R. King.** 1987. Overexpression of the EGF receptor-related proto-oncogene *erbB-2* in human mammary tumor cell lines by different molecular mechanisms. *EMBO J.* **6**:605-610.
 23. **Le, J., and J. Vilcek.** 1987. Tumor necrosis factor and interleukin 1: cytokines with multiple overlapping biological activities. *Lab. Invest.* **56**:234-248.
 24. **Muller, W. J., E. Sinn, P. K. Pattengale, R. Wallace, and P. Leder.** 1988. Single step induction of mammary adenocarcinoma in transgenic mice bearing the activated *c-neu* oncogene. *Cell* **54**:105-115.
 25. **Napier, M. A., M. T. Lipari, R. G. Courter, and C. H. K. Cheng.** 1987. Epidermal growth factor receptor tyrosine kinase phosphorylation of glucose-6-phosphate dehydrogenase *in vitro*. *Arch. Biochem. Biophys.* **259**:296-304.
 26. **Oi, V., and L. Herzenberg.** 1980. Immunoglobulin-producing hybrid cell lines, p. 351. *In* B. Mishel and S. Schiigi (ed.), *Selected methods in cellular immunology*. W. J. Freeman Co., San Francisco.
 27. **O'Toole, C. M., S. Povey, P. Hepburn, and L. M. Franks.** 1983. Identity of some human bladder cancer cell lines. *Nature (London)* **301**:429-430.
 28. **Patzer, E. J., G. R. Nakamura, and A. Yaffe.** 1984. Intracellular transport and secretion of hepatitis B surface antigen in mammalian cells. *J. Virol.* **51**:346-353.
 29. **Phillip, R., and L. B. Epstein.** 1986. Tumor necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, γ -interferon and interleukin-1. *Nature (London)* **323**:86-89.
 30. **Polanowski, G. P., E. V. Gaffney, and R. E. Burke.** 1976. HBL-100, a cell line established from human breast milk. *In Vitro (Rockville)* **12**:328.
 31. **Sato, J. D., T. Kawamoto, A. D. Le, J. Mendelsohn, J. Polikoff, and G. H. Sato.** 1983. Biological effects *in vitro* of monoclonal antibodies of human epidermal growth factor receptors. *Mol. Biol. Med.* **1**:511-529.
 32. **Sato, J. D., A. D. Le, and T. Kawamoto.** 1987. Derivation and assay of biological effects of monoclonal antibodies to epidermal growth factor receptors. *Methods Enzymol.* **146**:63-81.
 33. **Schechter, A. L., M.-C. Hung, L. Vaidyanathan, R. A. Weinberg, T. L. Yang-Feng, U. Francke, A. Ullrich, and L. Coussens.** 1985. The *neu* gene: an *erbB*-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. *Science* **229**:976-978.
 34. **Semba, K., N. Kamata, K. Toyoshima, and T. Yamamoto.** 1985. A *v-erbB*-related protooncogene, *c-erbB-2*, is distinct from the *c-erbB-1*/epidermal growth factor-receptor gene and is amplified in a human salivary gland adenocarcinoma. *Proc. Natl. Acad. Sci. USA* **82**:6497-6501.
 35. **Slamon, D. J., G. M. Clark, S. G. Wong, W. J. Levin, A. Ullrich, and W. L. McGuire.** 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. *Science* **235**:177-182.
 36. **Sugarman, B. J., B. B. Aggarwal, P. E. Hass, I. S. Figari, M. A. Palladino, and H. M. Shepard.** 1985. Recombinant human tumor necrosis factor- α : effects on proliferation of normal and transformed cells *in vitro*. *Science* **230**:943-945.
 37. **Sugarman, B. J., G. D. Lewis, T. E. Eessalu, B. B. Aggarwal, and H. M. Shepard.** 1987. Effects of growth factors on the antiproliferative activity of tumor necrosis factors. *Cancer Res.* **47**:780-786.
 38. **Urban, J. L., and H. Schreiber.** 1983. Selection of macrophage-resistant progressor tumor variants by the normal host. *J. Exp. Med.* **157**:642-656.
 39. **Urban, J. L., H. M. Shepard, J. L. Rothstein, B. J. Sugarman, and H. Schreiber.** 1986. Tumor necrosis factor: a potent effector molecule for tumor cell killing by activated macrophages. *Proc. Natl. Acad. Sci. USA* **83**:5233-5237.
 40. **van de Vijver, M., R. van de Bersselaar, P. Devilee, C. Cornelisse, J. Peterse, and R. Nusse.** 1987. Amplification of the *neu* (*c-erbB-2*) oncogene in human mammary tumors is relatively frequent and is often accompanied by amplification of the linked *c-erbA* oncogene. *Mol. Cell. Biol.* **7**:2019-2023.
 41. **Waldman, B. C., C. Oliver, and S. S. Krag.** 1987. A clonal derivative of tunicamycin-resistant Chinese hamster ovary cells with increased N-acetylglucosamine-phosphate transferase activity has altered asparagine-linked glycosylation. *J. Cell. Physiol.* **131**:302-317.
 42. **Yamamoto, T., S. Ikawa, T. Akiyama, K. Semba, N. Nomura, N. Miyajima, T. Saito, and K. Toyoshima.** 1986. Similarity of protein encoded by the human *c-erbB-2* gene to epidermal growth factor receptor. *Nature (London)* **319**:230-234.
 43. **Yarden, Y., and A. Ullrich.** 1988. Growth factor receptor tyrosine kinases. *Annu. Rev. Biochem.* **57**:443-478.
 44. **Yarden, Y., and A. Ullrich.** 1988. Molecular analysis of signal transduction by growth factors. *Biochemistry* **27**:3113-3119.
 45. **Yokota, J., T. Yamamoto, N. Miyajima, K. Toyoshima, N. Nomura, H. Sakamoto, T. Yoshida, M. Terada, and T. Suigimura.** 1988. Genetic alterations of the *c-erbB-2* oncogene occur frequently in tubular adenocarcinoma of the stomach and are often accompanied by amplification of the *v-erbA* homologue. *Oncogene* **2**:283-287.
 46. **Zhou, D., H. Battifora, J. Yokota, T. Yamamoto, and M. J. Cline.** 1987. Association of multiple copies of the *c-erbB-2* oncogene with spread of breast cancer. *Cancer Res.* **47**:6123-6125.