The heregulin-ErbB system of ligands and receptors are newly described epidermal growth factor (EGF) and EGF-receptor-related proteins that regulate growth, differentiation, and gene expression in numerous cell types. This study describes a receptor for heregulin β-1 (HRGβ1) on cultured rat hepatocytes and an inhibitory influence of insulin on HRGβ1 binding. HRGβ1 (30 nm) stimulated DNA synthesis 2-fold and was not augmented by insulin as is the case with EGF receptor ligands. A labeled peptide corresponding to the EGF domain of HRGβ1 bound to a single population of 19,600 ± 1,800 binding sites/cell with a Kd of 360 ± 22 pm. Cross-linking experiments showed binding of HRGβ1 to ErbB3 but not ErbB2 or ErbB4. HRGβ1 induced phosphorylation of ErbB3 and decreased ErbB3 protein levels, suggesting that HRGβ1 activates signaling through the ErbB3 receptor and influences receptor trafficking. Following plating, 125I-HRGβ1 binding and ErbB3 protein levels increased 8- and 3-fold, respectively, over the first 12 h in culture. These increases required de novo protein synthesis and were inhibited with 50 nm insulin resulting in 3500 binding sites with a Kd of 265 pm. These data suggest that the heregulin-ErbB system can regulate liver functions and may be linked to the metabolic and nutritional status of the animal.

Heregulins, also called neu differentiation factors or neu-regulins, are a family of EGF-like1 ligands that arise from differential splicing of transcripts from a single gene (1). A number of different biological activities have been associated with the various heregulin products. These activities include: (a) stimulation of mammary epithelial cell growth (2, 3), (b) stimulation of breast tumor cell growth (4–6), (c) breast tumor cell differentiation (7–10), (d) proliferation of Schwann cells (11, 12), (e) up-regulation of acetylcholine receptors in muscle cells (13), (f) regulation of neural crest cell development (13), and (g) regulation of Schwann cell precursor survival, proliferation, and maturation (14).

Heregulins signal through a family of EGF receptor-like tyrosine kinase receptors that include ErbB2 (neu), ErbB3 (HER3), and ErbB4 (HER4) (15). Several studies have suggested that heregulins are ligands for ErbB3 and ErbB4 (16) and that heterodimeric complexes of ErbB proteins form a high affinity heregulin binding site (17) and designate the signal transduction pathway(s) activated following binding (18, 19). Although heregulins do not bind the EGF receptor ligands do not bind ErbB2, ErbB3, or ErbB4, interactions between these receptors occur through inter-receptor transphosphorylations (15, 20).

Ligands of the EGF receptor, especially TGF-α, have been strongly implicated in the burst of compensatory hyperplasia following partial hepatectomy (21–25), a process that is also strongly influenced by other hormones, growth factors, and nutritional signals (26–30). Liver regeneration is a striking example of the synchronized proliferation of multiple cell types within a tissue that simultaneously maintains complex metabolic and other differentiated functions. The purpose of our study was to explore the possibility that the heregulins and their receptors are involved in the control of growth or differentiated functions in the liver. We demonstrate that a recombinant peptide corresponding to the EGF domain of the β-1 isoform of heregulin (HRGβ1) bound to rat hepatocytes via the ErbB3 receptor, induced receptor phosphorylation, and stimulated DNA synthesis. HRGβ1 treatment led to decreased levels of ErbB3 in freshly plated hepatocytes in a manner consistent with ligand-mediated down-regulation. Insulin inhibited the up-regulation of ErbB3 in vitro, suggesting a mechanism whereby metabolic controls could modulate proliferative and differentiative signals within liver.

**EXPERIMENTAL PROCEDURES**

Peptides, Reagents, and Radiochemicals—Human recombinant HRGβ1 (amino acids 177–244) was prepared as described (4). HRGβ1 was iodinated to a specific activity of 250–300 μCi/μg by the lactoperoxidase method (4). This protein corresponds to the EGF domain of the mature secreted form of HRGβ1 amino acids 177–244. Insulin was from Eli Lilly & Co. (Indianapolis, IN). Synthetic rat TGF-α was purchased from Peninsula Laboratories Inc. (Belmont, CA). [125-I]IGF-α was prepared using the chloramine T method as described (22, 31). [3H-methyl]Thymidine (6 Ci/mmol) was from DuPont NEN and was diluted to 0.36 Ci/mmol with cold thymidine. Thymidine, desamethasone, pyruvate, bovine serum albumin (fatty acid-free), percoll, bis(sulfosuccinimidyld)L-suberate, and all buffer reagents were from Sigma. Protein G-Sepharose was from Pierce, and ECL reagents and nitrocellulose membranes were from Amersham Corp. Anti-ErbB2 antibodies were from NeoMarkers (Fremont, CA). Anti-ErbB3 and anti-ErbB4 antibodies were from Santa Cruz (Santa Cruz, CA). Anti-EGRF antibodies were the kind gift of Dr. H. S. Earp.

Culture Media and Supplies—Williams’ Medium E, supplemented with 20 nm pyruvate, 10 nm desamethasone, and 50 μg/ml gentamicin, was the medium used for all culture studies including binding assays. Medium and calf serum were purchased from Life Technologies, Inc. Type I collagenase was from Wako Pure Chemical Industries, Ltd. (Richmond, VA) and Falcon six-well culture dishes were from Fisher.

Animals—Male Sprague-Dawley rats (150–200 g), from Harlan (In...
of stimulation, the cells were refed with medium containing specified
viable. Cells (375,000 cells/well) were plated in type-1 collagen-coated
hepatocytes were assessed for viability by trypan blue exclusion (≥ 95% viable). Cells (375,000 cells/well) were plated in type-1 collagen-coated

35-mm wells. After a 30-min attachment period, the serum-containing
medium was replaced with 1.5 ml of serum-free medium containing
growth factors as indicated.

DNA Synthesis Assays—Except where indicated otherwise, after 48 h of
serum-free culture, the cells were refed with medium containing specified
growth factors and 1 μCi/ml of [3H-methyl] thymidine (0.36 Ci/mmol).
At 72 h the experiment was terminated, and the incorporation of [3H-
ethyl] thymidine was determined. Cells were fixed and washed free of
unincorporated label with 5% trichloroacetic acid at 4°C. The cells were
then lysed in 1 N NaOH, and the DNA was precipitated by adding
 perchloric acid to a concentration of 1.5%. The DNA was hydrolyzed at
90°C for 15 min, and aliquots were taken for scintillation counting and
determination of DNA content using Richards's modification of the Bur-
Diphosphorylamine assay (34). The results of assays in triplicate are
expressed as the specific activity of the DNA (dpm/μg DNA ± S.D.).

In autoradiography studies, cells incorporated [3H]thymidine from
serum-free culture. After incubation, ascorbic acid (final) = 230
mM was added for 20 min at room temperature. Then the cells were
washed twice in methanol, rehydrated in deionized water, and overlaid
with photographic emulsion for 72 h before developing and counter-
staining with Giemsa. The results are represented as the percentage of
labeled nuclei from a total of at least 300 nuclei.

HRGβ1 Binding Studies—[125I]HRGβ1 binding to cultured hepatocy-
tes was assessed using a modification of our previous method for
[125I]EGF binding (32, 35). After the indicated times and conditions of
incubation, hepatocytes were placed at 4°C for 10 min. Medium was
then aspirated and replaced with ice-cold binding medium (Williams'
medium E containing 2 mg/ml bovine serum albumin, 20 mM HEPES,
then aspirated and replaced with ice-cold binding medium (Williams'
medium E containing 2 mg/ml bovine serum albumin, 20 mM HEPES,
[125I]HRGβ1 with or without a 50-fold molar excess of unlabeled HRGβ1
or EGF, respectively. Bis(sulfosuccinimidyl) suberate was then added to
a final concentration of 2 nM, and the cells were incubated for an
additional 40 min at room temperature. The reaction was stopped by
the addition of Tris-HCl to a final concentration of 50 mM. The cells
were then washed two times with phosphate-buffered saline and lysed
in TGH lysis buffer (with protease inhibitors). As a control, MCF-7 cells
were subjected to the same reaction conditions and analysis. Total
protein lysate (100 μg lane) was electrophoresed in a 6% acrylamide
gel, dried, and exposed to x-ray film. 400 μg of hepatocyte lysate was
incubated in separate reactions with the above anti-HRβ antibodies.
Immune complexes were precipitated with protein G, electrophoresed,
dried, and exposed to x-ray film.

Phosphorylation of ErbB3 by HRGβ1—Hepatocytes were cultured for
12 h and then stimulated for 5 min at 37°C with 300 nM HRGβ1 and
lysed in TGH with protease inhibitors (listed above) and phosphatase
inhibitors (10 mM sodium molybdate, 10 mM β-glycerol phosphate).
ErbB3 proteins were immunoprecipitated, transferred to membranes,
and blotted with anti-phosphotyrosine antibodies (RC20H, Transduction
Laboratories, Lexington, KY). Identical membranes were blotted with
anti-ErbB3 antibodies to normalize level of phosphorylation to amount
of receptor precipitated.

RESULTS

HRGβ1 Stimulates Hepatocyte DNA Synthesis—The combination
of EGF and insulin can stimulate 60–80% of rat hepatocytes
cultured in serum-free medium to undergo at least one
round of replicative DNA synthesis with a smaller number
actually undergoing mitosis (27, 38). Fig. 1 shows the effect of
increasing concentrations of TGF-α or HRGβ1 on [3H-methyl]
thymidine incorporation both alone and in the presence of 150
nM insulin. In contrast to ligands for the EGFr, the effects of
HRGβ1 were not appreciably amplified by the presence of 150
nM insulin. In a nuclear labeling assay, maximal stimulatory
doses of HRGβ1 (30 nM) stimulated 8.7 ± 0.84% of nuclei to
enter S phase by 72 h in culture as compared with 2.6 ± 0.83% of
control nuclei (data not shown). The stimulatory effects of
HRGβ1 alone and in the presence of 150 nM insulin were
completely inhibited by TGF-α (data not shown).

HRGβ1-Hepatocyte Binding Studies—Initial studies estab-
lished that binding equilibrium of [125I]HRGβ1 was reached by
4 h at 4°C (data not shown). The specificity of the HRGβ1
binding site in hepatocytes is shown in Fig. 2. Whereas the
binding of [125I]HRGβ1 was displaced by unlabeled HRGβ1,
500-fold molar excess concentrations of EGF, TGF-α, or amphi-
regulin, ligands for the EGFr, were ineffective. As reported by
Holmes et al. (4), a 500-fold excess of unlabeled HRGβ1 was
ineffective in displacing the binding of [125I]TGF-α from the
EGFr (data not shown). These results suggest a specific bind-
Pattern of recovery and decline in [125I]HRG EGFr.

The presence of insulin revealed that the diminished HRG was confirmed by the detection of the receptor in the rat hepatocyte. The sensitivity of the ErbB2 antibodies to rat protein immunodetectable in rat liver at 170 and 180 kDa, respectively. That of the proteins analyzed, only the EGFr and ErbB3 were present in rat liver, lysates of cultured hepatocytes andoma line H4IIe (data not shown).

Freshly isolated cells to 230,000 sites/cell 12 h after plating and then declining rapidly. The estimated EGFr receptor density at 12 h in culture corresponds well with independent estimates made on membranes from whole liver (40), suggesting that cell isolation results in a loss of EGFr that is transiently reversed over the first 12 h in culture. Fig. 3A shows a similar pattern of recovery and decline in [125I]HRG binding over time in culture. All subsequent estimates of HRG receptor number and affinity were performed on hepatocytes 12 h after plating.

To determine if the increase in [125I]HRG binding requires de novo protein synthesis or is regulated by physiologic signals, binding assays were performed on hepatocytes cultured for 12 h with or without 10 μg/ml cycloheximide or 150 nM insulin. Fig. 3B shows that the increase in [125I]HRG binding was blocked by cycloheximide and by insulin. Concentrations of insulin as low as 50 nM were able to completely inhibit the increase in HRG binding (data not shown).

Hepatocyte HRG1 binding sites could be saturated at 4°C.

Fig. 4 shows the binding of increasing amounts of [125I]HRG1 and the data resulting from Scatchard-type analysis using the Ligand program (37). A single population of 19,600 ± 1,800 binding sites per cell (mean ± S.D.) with a Kd of 360 ± 22 pm was identified in three separate hepatocyte preparations. Scatchard analysis of HRG1 binding to hepatocytes cultured in the presence of insulin revealed that the diminished HRG1 binding resulted from fewer binding sites of comparable affinity as control culture (Kd = 263 pm, sites/cell = 3532) (Fig. 4B).

Immunodetection of ErbB1 proteins in hepatocytes—To determine if the known components of the HRG1 receptor complex are present in rat liver, lysates of cultured hepatocytes and whole liver were immunoprecipitated with antibodies specific for rat EGFr, ErbB2, ErbB3, and ErbB4 receptors. Fig. 5 shows that of the proteins analyzed, only the EGFr and ErbB3 were immunodetectable in rat liver at 170 and 180 kDa, respectively. The sensitivity of the ErbB2 antibodies to rat protein was confirmed by the detection of the receptor in the rat hepatoma line H4IIe (data not shown).

HRG1-Hepatocyte Cross-linking and Immunoprecipitation—Following incubation with [125I]HRG1 or [125I]TGF-α as described above, ligand-receptor complexes were cross-linked with the cell-membrane impermeable coupling agent bis(sulfosuccinimidyl) suberate, lysed, and electrophoresed for autoradiography. As shown in Fig. 6A, [125I]HRG1 was associated with a single major molecular complex of approximately 180 kDa, and this association was completely inhibited in the presence of excess cold HRG1 to assess nonspecific binding. After incubation, the cells were washed, lysed, and counted to generate total () and nonspecific () binding curves. Specific binding () was calculated as total binding − nonspecific binding. B. A Scatchard-type plot generated by the Ligand program was compatible with a single population of 19600 ± 1800 sites/cell with a Kd of 360 ± 22 pm (C).

Effect of insulin, cycloheximide, and time in culture on HRG1 binding (Fig. 3A).

Effect of time in culture, insulin, and cycloheximide on HRG1 binding in hepatocytes. A, freshly isolated hepatocytes were incubated at 37 °C for 72 h. At the indicated timepoints, cells were chilled to 4 °C, incubated 4 h with 25 pm [125I]HRG1, and then washed and counted. B, hepatocytes were incubated in control medium (Con, ●), 150 nM insulin (Ins, ○), or 10 μg/ml cycloheximide (CHX, □) for 12 h. At the indicated time points, cells were chilled to 4 °C and incubated for 4 h with 1.5 nM [125I]HRG1 with or without a 200-fold excess of unlabeled HRG1 to correct for nonspecific binding. Radioactivity was analyzed as in A

Effect of insulin. A, cultured hepatocytes were incubated in growth factor free medium for 12 h then incubated with [125I]HRG1 at increasing concentrations for 4 h at 4 °C. Identical plates were prepared in the presence of excess cold HRG1 to assess nonspecific binding. After incubation, the cells were washed, lysed, and counted to generate total (○) and nonspecific (△) binding curves. Specific binding (●) was calculated as total binding − nonspecific binding. B, a Scatchard-type plot generated by the Ligand program was compatible with a single population of 19600 ± 1800 sites/cell with a Kd of 360 ± 22 pm (○). Control data are the means ± S.D. (n = 3 experiments). Effect of insulin on HRG1 binding (●). Fresh hepatocytes were incubated with 150 nM insulin for 12 h prior to Scatchard analysis. Kd = 263 pm, sites/cell = 3532.

Effect of cycloheximide. A, cultured hepatocytes were incubated in growth factor free medium for 12 h then incubated with [125I]HRG1 at increasing concentrations for 4 h at 4 °C. Identical plates were prepared in the presence of excess cold HRG1 to assess nonspecific binding. After incubation, the cells were washed, lysed, and counted to generate total (○) and nonspecific (△) binding curves. Specific binding (●) was calculated as total binding − nonspecific binding. B, a Scatchard-type plot generated by the Ligand program was compatible with a single population of 19600 ± 1800 sites/cell with a Kd of 360 ± 22 pm (○). Control data are the means ± S.D. (n = 3 experiments). Effect of cycloheximide on HRG1 binding (●). Fresh hepatocytes were incubated with 150 nM insulin for 12 h prior to Scatchard analysis. Kd = 263 pm, sites/cell = 3532.

Phosphorylation of ErbB3 in Hepatocytes by HRG1—After 12 h of incubation, hepatocytes were stimulated for 5 min with 300 nM HRG1 at 37 °C. Western blotting with anti-phosphotyrosine antibodies showed more than a 2-fold increase in the signal intensity at 170 kDa of ErbB3, which is the primary binding protein in rat hepatocyte HRG receptors.
phosphotyrosine content of ErbB3 stimulated with HRGβ1a compared with controls or insulin-treated ErbB3. (Fig. 7)

Effect of Growth Factors on ErbB3 Expression in Cultured Hepatocytes—Hepatocytes were incubated for 8 h with HRGβ1, insulin, or EGF. Figs. 8 and 9 show that within 8–12 h under control conditions, ErbB3 protein expression increased 2–3-fold, consonant with the observed increase in HRGβ1 binding. Both HRGβ1 (Fig. 8) and insulin (Fig. 9A) and to a lesser extent EGF (Fig. 8) inhibited the increase in ErbB3 protein levels. Neither insulin (Fig. 9B) nor HRGβ1 (data not shown) affected EGFr expression. In three separate hepatocyte preparations, HRGβ1 or insulin treatment resulted in ErbB3 expression that was 25 ± 2 or 32 ± 10% of controls, respectively. These results are consistent with a down-regulation of ErbB3 by HRGβ1 in a ligand-dependent manner and suggests that the depressed HRGβ1 binding induced by insulin results from a decrease in the expression of ErbB3 protein.

**DISCUSSION**

Heregulins elicit proliferation and/or differentiation in cells expressing ErbB receptors (20, 41). We questioned whether heregulin system of ligands and receptors is important in the control of liver growth and function. HRGβ1 at 30 nM stimulated DNA synthesis about 2-fold and was not augmented by insulin. This is in contrast to TGF-α, which at 10 nM stimulated a 30-fold increase in 3Hthymidine incorporation and was augmented at lower concentrations by insulin. Relative to TGF-α, which stimulates labeling of 80–85% of nuclei in cultured hepatocytes, HRGβ1 stimulated only 6% of the nuclei, suggesting possible compartmentalization of HRG-sensitive hepatocytes (42) and an underestimation of the number of binding sites per cell. Alternately, heregulins may not be primary mitogens in rat liver but could regulate differentiation during...
Hepatic Heregulin Receptors

Fig. 9. Inhibition of ErbB3 protein expression by insulin in cultured hepatocytes. Freshly isolated hepatocytes were incubated at 37°C for 12 h with (■) or without (□) 150 nM insulin (Ins). At the indicated time points, cells were lysed, immunoprecipitated, and Western blotted with anti-ErbB3 antibodies. B, the same lysates were immunoprecipitated and Western blotted with anti-EGFr antibodies. Films were scanned and quantified by densitometry. con, control.

development, maintenance of differentiated functions during regeneration, or metabolism in response to nutritional status.

Heregulin (HRG), a recently described ErbB ligand, has been purified from human breast cancer cells and has been shown to be a mitogen for hepatocytes in vitro, acting synergistically with glucagon, EGF, and other peptide growth factors to stimulate DNA synthesis in cultured hepatocytes and regenerating liver (27, 38, 50). Although HRGβ1-mediated DNA synthesis was not significantly augmented by insulin, the inhibitory effect of insulin on HRGβ1 expression and HRGβ1 binding in cultured hepatocytes was striking. Insulin could affect ErbB3 expression directly or down-regulate the receptor by inducing the secretion of a ligand. Insulin may promote the expression of ErbB3 protein and HRG binding in vivo because the concentrations of insulin effective in inhibiting ErbB3 expression were within physiological ranges detected in the portal vein (51, 52). We speculate that insulin, perhaps with other hormones and growth factors, may permit complex nutritional or metabolic controls to influence the intensity or duration of growth and differentiative signals generated by activated ErbB3 receptors.

Our purpose in this study was to describe the interaction of a recently described ErbB ligand, heregulin, with hepatocytes. This ligand has been purified from human breast cancer cells and ras-transformed rat fibroblasts, but transcripts have been detected in normal human liver as well (4). Here we show that the HRGβ1 isofrom of heregulin can stimulate DNA synthesis in rat hepatocytes and binds to ErbB3, but the current studies do not define the role played by this ligand-receptor system in liver physiology. As has been reported in the case of astrocytes in culture, heregulin may act as a survival and/or differentiation factor for hepatocytes (53). The signaling pathways and cellular responses that result from activation of ErbB3 are currently under study in this laboratory. These studies set the

EGFr to form an active signaling complex in hepatocytes: (a) the previously cited data indicating that heterodimers of ErbB2 and ErbB3 constitute high affinity HRG binding sites in transfected cells, whereas monomers of ErbB3 exhibit low affinity binding (17); (b) the demonstration that despite its homology to other tyrosine kinase receptors, ErbB3 has weak or nonexistent kinase activity due to an Asp→Asn substitution in the catalytic site of the otherwise highly conserved kinase domain (47); (c) the demonstration that ErbB3 is known to be a substrate of ErbB2, ErbB4, and the EGFr and can designate the signal transduction pathway(s) activated following ligand binding (15, 18, 19). For example, activation of insulin 1,4,5-trisphosphate kinase by EGF has been recently shown to be dependent upon the intermediacy of ErbB3, which contains seven repeats of the binding motif specific for the p85 subunit of insulin 1,4,5-trisphosphate kinase (48). In Ba/F3 cells transfected with EGFr and ErbB3, heregulin treatment resulted in phosphorylation of both receptors, suggesting that ErbB3 can be a substrate for the EGFr kinase in a heregulin-induced ErbB3/EGFr heterodimer (49). No data exist to speculate on the possible influence of the EGFr on ErbB3 affinity for HRG in heteromeric complexes. We were unable to demonstrate any high molecular weight species consistent with ErbB multimers from cross-linked hepatocyte lysates, presumably due to relatively low levels of receptor expression as compared with transformed or transfected cell lines. However, the data in Fig. 8 suggest that EGF may also influence ErbB3 expression in a fashion similar to HRGβ1, perhaps due to internalization of ErbB3 and EGFr heterodimers. Heterodimerization with EGFr may explain the mechanism of ErbB3 phosphorylation and high affinity binding in our model. Current studies aim to identify the other proteins involved in HRG binding and signaling in hepatocytes and other liver cells.

Insulin regulation of hepatic HRG binding and ErbB3 expression is of potential physiological significance. Insulin continuously bathes the liver through the portal circulation and is a mitogen for hepatocytes in vitro, acting synergistically with glucagon, EGF, and other peptide growth factors to stimulate DNA synthesis in cultured hepatocytes and regenerating liver (17, 38, 50). Although HRGβ1-mediated DNA synthesis was not significantly augmented by insulin, the inhibitory effect of insulin on HRGβ1 protein expression and HRGβ1 binding in cultured hepatocytes was striking. Insulin could affect ErbB3 expression directly or down-regulate the receptor by inducing the secretion of a ligand. Insulin may promote the expression of ErbB3 protein and HRG binding in vivo because the concentrations of insulin effective in inhibiting ErbB3 expression were within physiological ranges detected in the portal vein (51, 52). We speculate that insulin, perhaps with other hormones and growth factors, may permit complex nutritional or metabolic controls to influence the intensity or duration of growth and differentiative signals generated by activated ErbB3 receptors.

Our purpose in this study was to describe the interaction of a recently described ErbB ligand, heregulin, with hepatocytes. This ligand has been purified from human breast cancer cells and ras-transformed rat fibroblasts, but transcripts have been detected in normal human liver as well (4). Here we show that the HRGβ1 isoform of heregulin can stimulate DNA synthesis in rat hepatocytes and binds to ErbB3, but the current studies do not define the role played by this ligand-receptor system in liver physiology. As has been reported in the case of astrocytes in culture, heregulin may act as a survival and/or differentiation factor for hepatocytes (53). The signaling pathways and cellular responses that result from activation of ErbB3 are currently under study in this laboratory. These studies set the
groundwork for the clarification of the role of the heregulins and their receptors in the normal growth and function of the liver, and their possible involvement in the proliferative response to injury and partial hepatectomy, in the acute phase response, and in the development of hepatic neoplasia.

Acknowledgments—We thank Dr. H. S. Earp for the anti-EGFr antibody and Dr. R. J. Coffey for amphiregulin.

REFERENCES

1. Marchionni, M. A., Goodearl, A. D. J., Chen, M. S., Bermingham-McDonogh, D., Koca, C., Hendricks, M., Danely, F., Misumi, D., Sudhalter, J., Kobayashi, K., Wredebski, D., Lynch, C., Balassa, M., Hiles, I., Bavis, H. B., Hsuan, J. J., Totty, N. F., Otso, M., McBurney, R. N., Waterfield, M. D., Stroobant, P., and Gwynne, D. (1993). Nature 362, 312–318.

2. Marté, B. M., Graus-Porta, D., J. eschke, M., Fabbrö, D., Hynes, N. E., and Taverna, D. (1995) Oncogene 10, 167–175.

3. Ram, T. G., Kokeny, K. E., Dills, C. A., and Ethier, S. P. (1995) J. Cell. Physiol. 163, 589–596.

4. Holmes, W. E., Silwikowski, M. X., Akita, R. W., Henzel, W. J., Lee, J., Park, J. W., Yansura, D., Abad, N., Raab, H. G., Shepard, H. M., Kuang, H., Wood, W. I., Goeddel, D. V., and Vandlen, R. L. (1992) Science 256, 1205–1210.

5. Graus-Porta, D., Beeri, R. R., and Hynes, N. E. (1995) Mol. Cell. Biol. 15, 1182–1191.

6. Pietras, R. J., Arboleda, J., Reese, D. M., Wongvipat, N., Pegram, M. D., Fonken, L. K., Blanken, R. A., Kirk, C., Hendricks, M., Danehy, F., Misumi, D., Sudhalter, J., Kobayashi, K., Wredebski, D., Lynch, C., Balassa, M., Hiles, I., Bavis, H. B., Hsuan, J. J., Totty, N. F., Otso, M., McBurney, R. N., Waterfield, M. D., Stroobant, P., and Gwynne, D. (1993) Nature 362, 312–318.

7. Wallasch, C., Weilss, F. U., Niederfellner, G., J. allai, B., Issing, W., and Ulrich, A. (1995) EMBO J. 14, 4267–4275.

8. Peles, E., and Yarden, Y. (1993) Bioessays 15, 815–824.

9. Shoyab, M., Plowman, G. D., McDonald, V. L., Bradley, J. G., and Todaro, G. J. (1989) Science 243, 2148–2159.

10. Girardin, S., and Gahan, C. M. (1992) J. Cell. Biol. 119, 220–239.

11. Levi, A. D., Bunge, R. P., Lofgren, J. A., Meima, L., Hefti, F., Nikolics, K., and Silwikowski, M. X. (1995) J. Neurosci. 15, 1329–1340.

12. Falls, D. L., Rosen, K. M., Peggam, N., Pegram, M. D., Ramos, L., Gorman, C. M., Parker, M. G., Silwikowski, M. X., and Slamon, D. J. (1995) Oncogene 10, 2435–2446.

13. Hunter, W. M., and Greenwood, F. C. (1962) Nature 194, 495–496.

14. Dong, Z., Brennan, A., Liu, N., Yarden, Y., Lefkowitz, G., Mirsky, R., and J. esen, K. R. (1995) Nature 375, 585–590.

15. Earp, H. S., Drusano, G. L., Li, X., and Yu, H. (1995) Breast Cancer Res. Treat. 35, 115–132.

16. Carraway, K. L., III, and Cantley, L. C. (1994) Cell 78, 5–8.

17. Silwikowski, M. X., Schaefer, G., Akita, R. W., Lofgren, J. A., Fitzpatrick, V. D., U, N. S., Silbiger, S. M., Ben Lew, Y., and Lund, R. (1992) Cell 69, 559–572.

18. Fall, D. L., Rosen, K. M., Corfas, G., Lane, W. S., and W. B. Saunders, Philadelphia.

19. Hunter, W. M., and Greenwood, F. C. (1962) Nature 194, 495–496.

20. Wallasch, C., Weilss, F. U., Niederfellner, G., J. allai, B., Issing, W., and Ulrich, A. (1995) EMBO J. 14, 4267–4275.

21. Peles, E., and Yarden, Y. (1993) Bioessays 15, 815–824.