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Heterodimerization of the erbB-1 and erbB-2 Receptors in Human Breast Carcinoma Cells: A Mechanism for Receptor Transregulation†

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ABSTRACT: The erbB-1 and erbB-2 protooncogenes encode homologous membrane receptors that respectively bind epidermal growth factor (EGF) and a still incompletely characterized ligand. Binding of EGF to its receptor is known to increase tyrosine phosphorylation of the erbB-2/*neu* receptor in tumor cells. To investigate the mechanism of this transregulatory pathway, we analyzed the interactions between the two receptors in SKBR-3 human breast carcinoma cells. Chemical cross-linking of ^{125}I-labeled EGF revealed that the radiolabeled EGF receptor coimmunoprecipitates with the erbB-2/*neu* receptor. In addition a cross-linked species of 360-kdalton molecular mass is also coimmunoprecipitated. The formation of the latter species is absolutely dependent on the presence of EGF receptor and thus appears to represent a heterodimer of the erbB-1 and erbB-2 receptors. In vitro kinase reaction assays revealed that receptor heterodimerization is induced by EGF binding and leads to a dramatic increase in the self-phosphorylation capacity of the dimerized receptors. Moreover, analysis of living SKBR-3 cells suggested that most of the EGF-induced transregulation of the erbB-2/*neu* receptor is due to receptor heterodimerization. In conclusion, heterodimers of erbB-1 and erbB-2 receptors may provide a mechanism for dual transductory functions of growth factors of breast tumor cells.

The erbB-2/*neu* protooncogene (also called HER2) was originally discovered in rats through the potent oncogenic potential of a point mutant of the gene (Schechter et al., 1984; Bargmann et al., 1986a). The encoded protein is a 185-kdalton membrane protein with intrinsic tyrosine kinase activity (Akiyama et al., 1986; Stern et al., 1986) that shares extensive structural homology with the receptor for the epidermal growth factor (EGF;[1] Bargmann et al., 1986b; Yamamoto et al., 1986; Coussens et al., 1985). The human homologue of the gene is frequently overexpressed in human adenocarcinomas, and the overexpression correlates with poor prognosis (Slamon et al., 1987; Van de Vijver et al., 1988; Berger et al., 1988; Slamon et al., 1989). Despite the homology to erbB-1/EGF receptor, the erbB-2/*neu* product does not bind EGF but appears to have a distinct ligand (Yarden & Weinberg, 1989). Activation of the tyrosine kinase function of the p185^{neu} protein is essential for cellular transformation by the oncogenic receptor (Bargmann & Weinberg, 1988a). The latter differs from the protooncogenic form by a single amino acid within the transmembrane domain of the protein. This change is associated with high tyrosine kinase activity in vitro (Bargmann & Weinberg, 1988b) and in living cells (Yarden, 1990) and appears to lead to the formation of constitutive receptor dimers (Weiner et al., 1989). Experiments done with monoclonal antibodies to p185^{neu} (Yarden, 1990) and with chimeric EGF receptor/*neu*-HER2 protein (Lehvaslaiho et al., 1989; Lee et al., 1989) demonstrated that the kinase function of the protooncogenic p185^{neu} can be allosterically regulated by antibody or ligand binding to the extracellular domain. Another way to increase tyrosine phosphorylation of the erbB-2/*neu* protein is through the binding of EGF to its receptor, which then transregulates p185^{neu} (Stern & Kamps, 1988; King et al., 1988; Kokai et al., 1988). Recently, these interactions were shown to lead to transformation of rodent fibroblasts (Kokai et al., 1989).

The present study addressed the molecular mechanism of transregulation of p185^{neu} by the occupied EGF receptor. On the basis of the observation that activation of the EGF receptor tyrosine kinase involves receptor dimerization (Yarden & Schlessinger, 1987a,b; Boni-Schenetzler & Pilch, 1987; Cochet et al., 1988), we assumed that a similar mechanism underlies transactivation of the erbB-2/*neu* kinase. Here we present results that experimentally support this hypothesis.

Materials and Methods

Materials. EGF was supplied by Biomakor and by Toyobo. Radioactive materials were purchased from Amersham. Protein A coupled to Sepharose was obtained from Pharmacia or prepared in our laboratory. Molecular weight standards for gel electrophoresis were from Bio-Rad. All other chemicals were purchased from Sigma, unless otherwise stated.

Cells. Human breast carcinoma cells (SKBR-3) were cultured in DME medium supplemented with 10% heat-inactivated fetal calf serum. CHO cells overexpressing p185^{neu} (HCC) were grown in F12 medium supplemented as above.

Buffered Solutions. HNTG buffer contained 20 mM HEPES (pH 7.5), 150 mM NaCl, 0.1% (w/v) Triton X-100, and 10% glycerol. Solubilization buffer contained 50 mM HEPES, pH 7.5, 150 mM NaCl, 1% (w/v) Triton X-100, 10%

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[1] Abbreviations: CHO, Chinese hamster ovary; DME, Dulbecco's modified Eagle's medium; EDAC, 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide; EGF, epidermal growth factor; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PBS, phosphate-buffered saline; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate.

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glycerol, 1.5 mM $MgCl_2$, 1 mM EGTA, aprotinin (0.15 trypsin inhibitor unit/mL), 1 mM PMSF, and 10 μg/mL leupeptin. PBS contained 137 mM NaCl, 2.7 mM KCl, 7.9 mM NaH_2PO_4, and 1 mM KH_2PO_4, pH 7.2. High (H′) wash contained 50 mM HEPES (pH 7.5), 500 mM NaCl, 0.1% SDS, 0.2% (w/v) Triton X-100, 5 mM EGTA, 20 mM NaF, 5 mM EDTA, and 2 mM NaV. Medium (M′) wash contained 50 mM HEPES, pH 7.5, 150 mM NaCl, 0.1% SDS, 0.2% (w/v) Triton X-100, 5 mM EGTA, 20 mM NaF, 5 mM EDTA, and 2 mM NaV. Low (L′) wash contained 10 mM Tris (pH 8.0), 0.1% (w/v) Triton X-100, 20 mM NaF, 5 mM EDTA, and 2 mM NaV.

Antibodies. A polyclonal antiserum to phosphotyrosine (PT5) was generated as described by Kamps and Sefton (1988). The NCT antiserum was raised in rabbits injected with a synthetic peptide that corresponds to the 16 C-terminal amino acids of human p185^{neu}. A similar antibody (Ab2) was generated against a synthetic peptide of the EGF receptor as described (Kris et al., 1985). The monoclonal antibodies R1, 108, and 225 to EGF receptor were previously described (Waterfield et al., 1982; Lax et al., 1989; Kawamoto et al., 1983). A monoclonal antibody to human p185^{neu} (Ab29) was recently generated in our laboratory with SKBR-3 cells as an immunogen.

In Vivo Stimulation and Cross-Linking on Living Cells. Cells were grown to confluence in 15-cm dishes. The monolayers were then washed once with cold PBS on ice, and fresh PBS with EGF (100 ng/mL) was applied to the cells and allowed to incubate at 4 °C for 1 h. Cross-linker (EDAC in PBS, 15 mM) was added to the cultures for 45 min at 22 °C. Cells were then returned to ice and washed twice with PBS. In some experiments we used radiolabeled EGF that was prepared by the chloramine T method as described (Hock & Hollenberg, 1980).

Lysate Preparation and Immunoprecipitation. Solubilization buffer was added to the monolayer of cells on ice. Cells were scraped with a rubber policeman into 1 mL of the buffer, transferred to microtubes, vortexed harshly, and centrifuged (600*g*, 10 min at 4 °C). Antibodies were coupled to protein A–Sepharose while shaking for 20 min. The proteins in the lysate supernatants were immunoprecipitated with aliquots of the protein A–Sepharose–antibody complex for 1 h at 4 °C. Immunoprecipitates were then washed three times with HNTG (1 mL each wash).

SDS–Polyacrylamide Gel Electrophoresis (SDS–PAGE). Standard slab gels of 1-mm thickness were used. Gels were of 4.5% acrylamide density with a stacking gel of 3.5%. Samples to be electrophoresed were mixed with concentrated electrophoresis buffer and were heated to 95 °C for 5 min.

In Vitro Phosphorylation Assay. Washed immunoprecipitates were resuspended in 0.05 mL of HNTG that contained $MnCl_2$ (10 mM) and [γ-^{32}P]ATP (3 μCi/reaction tube) and incubated for 15–20 min on ice. The samples were then washed sequentially with H′, M′, and L′ solutions (1 mL each wash) and subjected to gel electrophoresis.

Western Blotting. Immunoprecipitates were subjected to gel electrophoresis. The proteins were transferred onto a nitrocellulose sheet at 200 mA for 1–2 h. The nitrocellulose was blocked with 1% hemoglobin for 2 h, followed by overnight incubation (4 °C) with affinity-purified antibody (goat) to rabbit IgG (15 μg/mL, in Tris-buffered saline containing 1% hemoglobin). The blot was then washed with Tris-buffered saline with 0.1% Triton X-100 (TBSX), three times for 15 min each wash. ^{125}I-Labeled protein A in 1% hemoglobin was allowed to react with the blot for 1 h at 22 °C. A further three washes, as above, were done, and the blot was exposed to film.

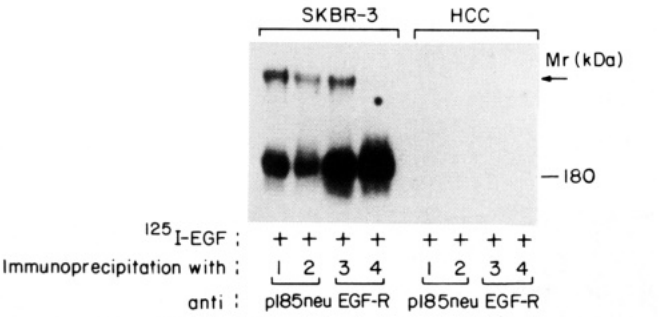

FIGURE 1: Cross-linking of ^{125}I-EGF to erbB-1 and erbB-2 receptors. ^{125}I-EGF (100 ng/mL, 100 000 cpm/ng) was added to confluent monolayers of SKBR-3 or HCC cells. Following 1 h of incubation at 4 °C in PBS, the cells were subjected to chemical cross-linking with EDAC (15 mM, 45 min at 22 °C). Cell lysates were then prepared and immunoprecipitated with the following antibodies: A polyclonal antibody to p185^{neu} (NCT-30, lane 1), a monoclonal antibody to p185^{neu} (Ab29, lane 2), a polyclonal antibody to EGF receptor (Ab2, lane 3), and a monoclonal antibody to EGF-R (Ab225, lane 4). The washed immunoprecipitates were resolved by gel electrophoresis. An autoradiogram of 12-h exposure is shown, and the location of the dimeric form is indicated by an arrow.

Ligand-Induced Downregulation. Confluent monolayers of SKBR-3 cells were incubated for various time intervals with 20 ng/mL EGF at 37 °C (initial control with no EGF was also taken). This incubation was followed by 1 h at 4 °C with ^{125}I-EGF (100 ng/mL) and a further 45 min at 22 °C with EDAC (15 mM). Immunoprecipitation was carried out as described above, followed by gel electrophoresis.

In Vivo Phosphorylation Assay. Cells were stimulated as described in the text. Lysates were immunoprecipitated with anti-phosphotyrosine antibodies (PT5) for 30 min at 4 °C. After being washed three times with HNTG, the precipitated proteins were specifically eluted into solubilization buffer that contained phenyl phosphate (50 mM) by shaking for 15 min at 4 °C. This eluant was then immunoprecipitated with anti-receptor antibodies during a 30-min incubation. The beads were washed three times with HNTG and then reacted with [γ-^{32}P]ATP, as described above. After washing, the samples were subjected to gel electrophoresis.

Results

Antibodies to erb-2/neu Receptor Precipitate ^{125}I-EGF-Cross-Linked Proteins. To study protein–protein interactions involving the erbB-1 and erbB-2 proteins, we specifically labeled the erbB-1/EGF receptor to high specific activity using chemical cross-linking of ^{125}I-EGF. The cellular system employed was the SKBR-3 human breast carcinoma cell line that overexpresses the erbB-2 receptor and also expresses the erbB-1/EGF receptor, but at a much lower level (Kraus et al., 1987). These cells exhibit EGF-induced modification of the erbB-2 receptor (King et al., 1988). Utilizing antibodies to the erbB-1/EGF-receptor, we immunoprecipitated two labeled proteins from lysates of SKBR-3 cells that were pretreated with ^{125}I-EGF and a cross-linking reagent (Figure 1). These 180- and 360-kdalton proteins were identified as EGF receptor monomer and dimer forms, respectively, on the basis of previous analyses (Cochet et al., 1987). Interestingly, monoclonal antibodies (225 and 108) that recognize the ligand binding domain of EGF receptor, but not the R1 antibody, did not react with the dimeric form. Importantly, monoclonal and polyclonal antibodies to the erbB/*neu* protein, but not control mouse or rabbit antibodies (not shown), precipitated protein bands of similar molecular masses (Figure 1). It is worth to note that these two different antibodies immuno-

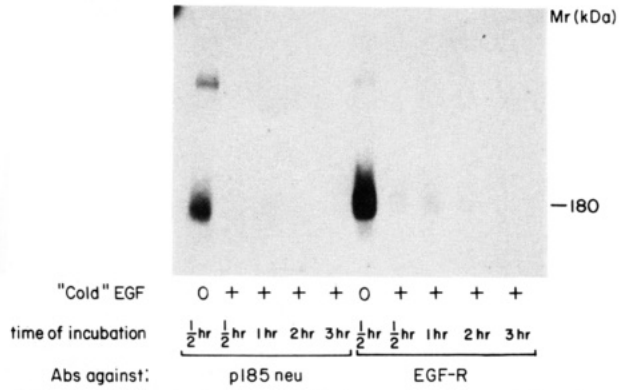

FIGURE 2: Downregulation of both the monomeric and dimeric receptor forms by preincubation with EGF. Confluent monolayers of SKBR-3 cells were incubated at 37 °C in PBS in the absence (0) or presence (+) of EGF (20 ng/mL). After the indicated periods of time the monolayers were washed extensively and then incubated with ^{125}I-EGF (100 ng/mL) for 1 h at 4 °C, followed by the addition of EDAC and further incubation (45 min at 22 °C). Cell lysates were prepared and immunoprecipitated with antisera directed to p185^{neu} (NCT antibody) or EGF receptor (Ab2), as indicated. An autoradiogram (17-h exposure) of the gel-resolved immunoprecipitates is shown.

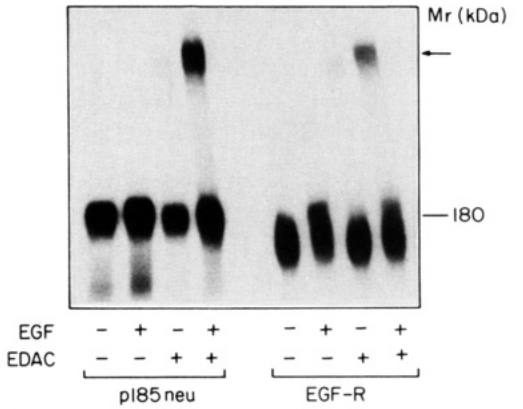

FIGURE 3: Effect of EGF on heterodimerization by an in vitro kinase assay. SKBR-3 cells (4×10^5) were incubated for 1 h at 4 °C in the absence or presence of EGF (100 ng/mL). The monolayers were then transferred to 22 °C, and a chemical cross-linker (EDAC) was added as indicated. After an additional hour cell lysates were prepared and proteins immunoprecipitated with anti-p185^{neu} antibodies (NCT) or Ab2 to EGF receptor, as indicated. The immunoprecipitates were then subjected to phosphorylation in vitro as described under Materials and Methods. An autoradiogram (16-h exposure) of the gel-separated immunocomplexes is shown, and the location of the dimeric forms is indicated with an arrow.

precipitated much less of the 180-kdalton protein as compared with antibodies to EGF receptor. However, the extents of precipitation of the 360-kdalton band which was also slightly upshifted were comparable to that observed with EGF receptor antibodies. The simplest explanation to the pattern seen with antibodies to erbB-2/*neu* protein is that this receptor, which is present in large excess, interacts with the related receptor for EGF to form noncovalently held heterodimers. Accordingly, the coprecipitated EGF receptor monomers are due to failure of the chemical cross-linking to stably associate these two receptors one with the other, whereas the dimeric form represents chemically cross-linked heterodimers of the EGF receptor (p170) and the erbB-2/*neu* receptor (p180).

Alternatively, given the high homology between erbB-1 and erbB2 (Coussens et al., 1985), it could be that the chemical cross-linker stabilized the otherwise very weak interactions between EGF and the erbB-2/*neu* protein. To exclude this possibility, we examined the dependency of the coprecipitation on the presence of EGF receptor. Chinese hamster ovary cells, which do not express the erbB-1/EGF receptor gene, were transfected with a plasmid vector that directs the expression of the erbB-2/HER2 gene. A single cell clone that expresses $(5–7) \times 10^5$ erbB-2/HER2 receptor was selected and designated HCC (E. Peles, A. Ullrich, and Y. Yarden, submitted for publication). Cross-linking of ^{125}I-EGF to these cells resulted in no immunoprecipitation of labeled proteins (Figure 1), indicating that the presence of EGF receptor is absolutely required for the coimmunoprecipitation effect. The same conclusion was reached with SKBR-3 cells from which EGF receptors were downregulated by a prior exposure to low concentrations of unlabeled EGF (Figure 2). Again, no cross-linked protein bands were seen with SKBR-3 cells after downregulation of the EGF receptor. This result cannot be explained by direct low-affinity interactions between EGF and the erbB-2/*neu* receptor. We therefore conclude that the 360-kdalton protein band that undergoes immunoprecipitation with antibodies to erbB-2/*neu* receptor is a heterodimer of an EGF receptor and an erbB-2 protein.

Detection of an EGF-Induced Heterodimer by in Vitro Kinase Assay. In order to examine the EGF inducibility and kinase activity of the heterodimeric receptor form of erbB-2, we performed in vitro kinase assays. SKBR-3 cells were incubated in the presence or absence of EGF and then subjected to cross-linking by EDAC. The EGF receptor and the erbB-2/*neu* protein were then immunoprecipitated, and their kinase activity was analyzed by the addition of [γ-^{32}P]ATP. As shown in Figure 3, the addition of EGF resulted in the appearance of phosphorylated bands of 360 kdaltons that could be immunoprecipitated with both antibodies to EGF receptor and antibodies to the erbB-2/*neu* protein. No other high molecular mass proteins could be detected. On the basis of the data shown in Figure 1, we assumed that the 360-kdalton protein precipitated by antibodies to erbB-2/*neu* is a heterodimer of the erbB-1 and erbB-2 gene products. As no heterodimer exists in the absence of EGF or the cross-linking agent, we concluded that no basal heterodimerization occurs and that only noncovalent interactions stabilize the heterodimeric structure.

The ratio of dimer to monomer phosphorylation appears to be significantly higher in the case of the heterodimeric form than in the case of dimer form obtained with anti-EGF receptor antibodies (Figure 3). This may suggest that heterodimerization of the erbB-1 and erbB-2 kinases involves higher catalytic activation than homodimerization of erbB-1 receptors. In order to further examine this possibility, we attempted to determine the relative kinase specific activities of the monomeric and dimeric forms of erbB-2. To this end, we performed Western blot analysis of SKBR-3 cells treated with EGF and EDAC. It is evident from Figure 4 that the heterodimers that are visible by functional assays (i.e., cross-linking of ^{125}I-EGF and in vitro kinase assays) are below the level of detection by Western blot analysis. The same conclusion was reached in experiments in which we attempted to detect [^{35}S]methionine-labeled heterodimers (data not shown). Conceivably then, the heterodimers caught by cross-linking are present at extremely low levels, but their kinase activity and presumably also the ligand binding function are very high as compared with these activities of the monomeric erbB-2 receptor.

Ligand-Induced Heterodimerization Is Associated with Increased Tyrosine Phosphorylation of erbB-2 in Living SKBR-3 Cells. We next addressed the possibility that the EGF-induced heterodimerization leads to increased receptor self-phosphorylation in living cells. Our attempts to approach

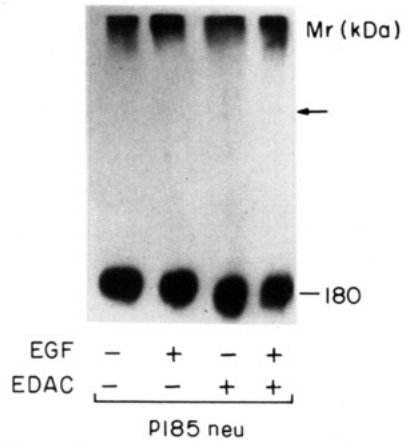

FIGURE 4: Western blot analysis of chemically cross-linked p185^{neu}. SKRB-3 cells were grown to confluence in 9-cm dishes and treated with EGF and EDAC as described in the legend to Figure 3. Cell lysates were subjected to immunoprecipitation with an antiserum to p185^{neu} (NCT antibody), and the immunocomplexes were resolved by electrophoresis in a 4.5% acrylamide gel. Proteins were then electrophoretically transferred onto nitrocellulose and blotted with the NCT antiserum (1:500 dilution) followed by ^{125}I-labeled protein A (400 000 cpm/mL). The resulting autoradiogram (24-h exposure) is shown, and the predicted position of the dimer band is indicated by an arrow.

this experiment by employing Western blot analysis with phosphotyrosine antibodies or [^{32}P]orthophosphate-labeled cells failed due to low assay sensitivities. To overcome it, we established a procedure that enables dimer detection due to its tyrosine phosphorylation while in the living cell. SKBR-3 cells were treated with or without EGF and cross-linker (EDAC), and phosphotyrosine-containing proteins precipitated with specific antibodies. Specifically adsorbed proteins were then eluted by means of a phosphotyrosine analogue and subjected to a second immunoprecipitation step with antibodies either to erbB-2 or to EGF receptor. Finally, the washed immunocomplexes were incubated with [γ-^{32}P]ATP and Mn^{2+} to allow receptor self-phosphorylation. This final reaction is carried out for a relatively long period of time to allow complete saturation of the phosphorylation sites. It is known that in vitro phosphorylation of receptor tyrosine kinases takes place on multiple sites, at least part of which are not utilized in the living cell (Van der Geer & Hunter, 1990). Thus, the level of self-phosphorylation is presumably directly proportional to the extent of receptor tyrosine phosphorylation in the living cell. Indeed, preliminary assays done with a ligand-stimulated EGF receptor confirmed this assumption (data not shown). The results of this experiment are shown in Figure 5. Evidently, no basal tyrosine phosphorylation of erbB-2 and erbB-1 proteins occurred in living SKBR-3 cells. Upon addition of EGF both receptors underwent phosphorylation on tyrosine residues that is accompanied by the appearance of dimeric receptor forms. Comparison of the extent of labeling of the monomer and the dimer forms indicates that a significant proportion of the tyrosine modification of the erbB-2 protein takes place while in the dimer form. This ratio is less significant in the case of the erbB-1/EGF receptor. Considering the low efficiency of the cross-linking reaction, it is likely that most of the EGF-induced phosphorylation of the erbB-2 protein occurs while in the heterodimeric state or in oligomers of higher order.

Discussion

The EGF receptor and the transforming p185^{neu} protein were previously shown to undergo homodimerization (Yarden & Schlessinger, 1987a,b; Boni-Schenetzler & Pilch, 1987;

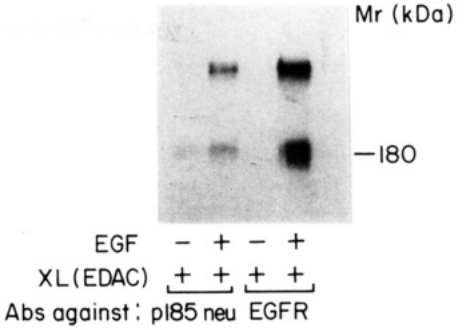

FIGURE 5: Tyrosine phosphorylation of erbB-1 and erbB-2 dimeric forms in living SKBR-3 cells. Confluent monolayers of SKBR-3 cells in 9-cm dishes were treated with EGF and a chemical cross-linker as described in the legend to Figure 3. Lysates were prepared and immunoprecipitated with rabbit antibodies to phosphotyrosine. Adsorbed phosphotyrosine proteins were eluted specifically with phenyl phosphate and subjected to a second immunoprecipitation with antisera to p185^{neu} (NCT antibody) or to EGF receptor (Ab2). The washed immunoprecipitates were assayed by in vitro phosphorylation and then resolved by gel electrophoresis and autoradiography (20-h exposure).

Cochet et al., 1988; Weiner et al., 1989). In the present study we have shown that these receptors also form heterodimers. The latter are held by noncovalent interactions, and their formation absolutely depends on the binding of EGF to its own receptor. Our data also provide evidence that the heterodimers are characterized by a dramatically increased capacity to undergo self-phosphorylation, and they probably bind EGF tighter than the monomeric species. In living cells the heterodimer probably accounts for most, if not all, of the ligand-induced tyrosine phosphorylation and thus appears to provide a mechanistic basis for receptor transregulation.

Greene and his colleagues (Wada et al., 1990) have recently reported on similar heterodimerization in murine fibroblasts that overexpress, as a result of molecular transfection, the human erbB-1 and the rat *neu*/erbB-2. Their results suggest that the heterodimer form confers a very high EGF-binding affinity and also mitogenic responsiveness to relatively low concentrations of the ligand. Our results were obtained with a more natural cellular system, namely, human breast carcinoma cells. This cellular system imposed technical problems due to the large excess (approximately 20-fold) of the erbB-2 protein over the EGF receptor. Nevertheless, our results are consistent with the data obtained in transfected murine fibroblasts. For example, we were not able to detect the heterodimer form by structural methods (Western blotting or metabolic labeling) due to the relatively low expression of EGF receptor in SKBR-3 cells. As a result of this difficulty, the heterodimeric receptor was identified solely on the basis of its function as a receptor for EGF and as a tyrosine kinase. On the basis of the ratio between the heterodimer and the monomer forms in Western blotting (Figure 4), kinase activity (Figure 3), and EGF binding (Figure 1), we concluded that the dimeric form is a much more active kinase and may bind EGF tighter than the monomeric form. However, we have not directly determined the affinity of each receptor species. It was previously shown that these are also the functional characteristics of the EGF receptor homodimers (Yarden & Schlessinger, 1987a,b; Boni-Schenetzler & Pilch, 1987). Thus, the homodimers and heterodimers of EGF receptor are functionally analogous. In addition, homodimers of the p185^{neu} which are induced by bivalent but not monovalent monoclonal antibodies were shown to involve increased receptor phosphorylation in living cells (Yarden, 1990). It was further suggested that constitutive homodimerization of the transforming mutant of p185^{neu} leads to persistent kinase activation

(Stern et al., 1988; Weiner et al., 1988; Sternberg & Gullick, 1990; Yarden, 1990). Thus, receptor interactions that are mediated by either homo- or heterodimerization appear to lead to kinase activation (Schlessinger, 1988). One mechanism that could underly this process is transphosphorylation within the dimeric structure (Honegger et al., 1988). Interestingly, it appears that the same sites of phosphorylation of p185^{neu} are affected in homodimerized and heterodimerized p185^{neu} (Yarden, 1990). Conceivably then, signals due to the binding of EGF or the ligand of the erbB-2/*neu* receptor may be presented to the cytoplasm in a functionally equivalent form. If correct, the heterodimerization mechanism may allow each ligand to act dually via two distinct receptors that are presumably linked to different signal transductory pathways (Lehvashiho et al., 1989; Lee et al., 1989). Such a mechanism may confer a selective advantage to cells that express both erbB-1 and erbB-2 receptors. Hence, the demonstration of heterodimerization in human breast carcinoma cells, which often express both receptors (Kraus et al., 1987), may have implications to tumor development.

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Registry No. EGF, 62229-50-9; EGF receptor tyrosine kinase, 79079-06-4; protein tyrosine kinase, 80449-02-1.

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