ELSEVIER
ACADEMIC
PRESS

Advances in PROTEIN CHEMISTRY

VOLUME 68

Cell Surface Receptors

ADVANCES IN PROTEIN CHEMISTRY

Volume 68

Cell Surface Receptors

This Page Intentionally Left Blank

ADVANCES IN PROTEIN CHEMISTRY

EDITED BY

FREDERIC M. RICHARDS
Department of Molecular Biophysics
and Biochemistry, Yale University
New Haven, Connecticut

DAVID S. EISENBERG
Department of Chemistry and Biochemistry
Center for Genomics and Proteomics
University of California, Los Angeles
Los Angeles, California

JOHN KURIYAN
Department of Molecular and Cellular Biology
University of California, Berkeley
Berkeley, California

VOLUME 68

Cell Surface Receptors

EDITED BY

K. Christopher Garcia
Stanford University School of Medicine
Stanford, California

ELSEVIER
ACADEMIC
PRESS

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Elsevier Academic Press
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
84 Theobald's Road, London WC1X 8RR, UK

This book is printed on acid-free paper. ♾

For all information on all Academic Press publications
visit our Web site at www.academicpress.com

ISBN: 0-12-034268-5

PRINTED IN THE UNITED STATES OF AMERICA
04 05 06 07 08 9 8 7 6 5 4 3 2 1

CONTENTS

Shared Cytokine Signaling Receptors: Structural Insights from the gp130 System

MARTIN J. BOULANGER AND K. CHRISTOPHER GARCIA

The Structural Basis for Biological Signaling, Regulation, and Specificity in the Growth Hormone–Prolactin System of Hormones and Receptors

ANTHONY A. KOSSIAKOFF

Structural Analysis of IL-10 and Type I Interferon Family Members and Their Complexes with Receptor

MARK R. WALTER

Assembly of Post-Receptor Singnaling Complexes for the Tumor Necrosis Factor Receptor Superfamily

HAO WU

NKG2D and Related Immunoreceptors

ROLAND K. STRONG AND BENJAMIN J. MCFARLAND

Ionotropic Glutamate Receptor Recognition and Activation

ROBERT E. OSWALD

Chemokine-Receptor Interactions: GPCRs, Glycosaminoglycans and Viral Chemokine Binding Proteins

ELAINE K. LAU, SAMANTHA ALLEN, ANDRO R. HSU, AND TRACY M. HANDEL

Chemotaxis Receptors and Signaling

AARON F. MILLER AND JOSEPH J. FALKE

This Page Intentionally Left Blank

PREFACE

Receptors are the gateway through which our cells sense and respond to their changing environments. Cell surface receptors have the unique capacity to both engage exracellular ligands, as well as relay this information through the plasma membrane to intracellular components, and beyond. Some receptors, such as growth factor and cytokine receptors, contain only a single-transmembrane segment, connecting modular extracellular and intracellular domains that can be studied as soluble fragments retaining full ligand-binding activity. Other receptors, such as G-protein coupled receptors are multi-pass transmembrane proteins requiring a lipid bilayer for functional reconstitution. Others systems, exemplified here by the glutamate receptor, are combinations of autonomous ligand binding extracellular domains connected to multiple transmembrane helix polypeptide cores. Regardless of structural classification, the fundamental question persists, how is ligand engagement outside the cell coupled to receptor activation within the cell? This question remains elusive even for the most intensively studied systems, and remains one of the most challenging problems in biochemistry. Structural biology has made great strides in understanding this question through the determination of structures of receptors and their complexes with ligands. Ultimately a clear elucidation of a detailed mechanistic picture of receptor activation will require a combination of static biophysical methods such as x-ray crystallography, with dynamic methods such as NMR and single-molecule spectroscopy, which together can be dovetailed with functional studies.

While the structural database now has many examples of protein-protein complexes which have taught us about the first principles of molecular recognition, receptors remain somewhat enigmatic in that they are protein machines, rather than simple binding proteins. The chemistry of receptor-ligand recognition is part of an overall process by which ligand orients, or otherwise perturbs the structure of a receptor in such a way that the intracellular adaptor proteins initiate signaling cascades. The basis by which this phenomenon occurs appears far more complex than we originally thought based on simple models of receptor homodimerization. Not so long ago there was a common assumption that simply bringing receptors together is all one needs for activation. More recently, it is being appreciated that subtle orientational differences in the extracellular domains of dimerized receptors can translate into very significant downstream signaling differences. Thus, the transmembrane helices may

not simply be loose tethers between the extracellular and intracellular domains. Rather, in a number of receptors systems such as Erythropoetin, the connecting peptides and transmembrane helices appear capable of transmitting torque though the lipid bilayer to effect orientational strain in the intracellular parts of the receptors. It is a fact that most cell surface receptors are oligomerized in some fashion by their ligands, but the geometric and conformational details of this clustering are critical to the appropriate signals being transduced. Examples exist now of ligands inducing large-scale conformational changes in preformed receptors dimers, as well as receptor activation through disruption of dimerization. Most recently, GPCR are now being shown to require some form of ligand-induced dimerization, in concert with conformational change of the helices, for activation. Given the technical challenges inherent in studying receptors at a biophysical level, it is likely we have currently seen only a small fraction of the universe of potential mechanisms for receptor activation.

This issue of Advances in Protein Chemistry presents detailed chapters on several important receptor systems, with an emphasis on relating the structural basis of extracellular ligand recognition to the activation of intracellular signaling events. Some chapters focus on structural aspects of ligand recognition, while others are more focused on mechanistic questions. However in all cases there is an attempt to paint a structural portrait for how ligand engagement may activate the receptor. We have emphasized systems for which a significant amount of structural information is known on either the extracellular or intracellular regions of the receptors. In most cases the chemistry of ligand recognition relates in subtle, yet still unclear ways to receptor activation. The receptors discussed in this edition range from type-I receptors for growth factors hormones, and immunoregulatory ligands (Leahy, Nikolov, Garcia, Kossiakoff, Walter, Wu, Springer, Strong) to multi-pass integral membrane proteins (Oswald, Falke, Handel). For several of these systems structures are known for both extracellular domains and intracellular domains, allowing models to be constructed for the entire receptor. Clearly, these all-encompassing models are an important future direction for the field of receptor structural biology.

I wish to thank all of the authors who contributed to this edition, and to the editorial staff and senior editors for making this a pleasant experience for all involved.

K. Christopher Garcia
Stanford University School of Medicine

STRUCTURE AND FUNCTION OF THE EPIDERMAL GROWTH FACTOR (EGF/ERBB) FAMILY OF RECEPTORS

By DANIEL J. LEAHY

Department of Biophysics & Biophysical Chemistry and HHMI,
Johns Hopkins University School of Medicine,
Baltimore, Maryland 21205

I. Introduction

Epidermal Growth Factor (EGF) was among the first growth factors discovered, and study of EGF and its receptors has established many paradigms for growth factor–mediated signaling (Carpenter, 1987; Cohen, 1986, 1987; Schlessinger, 2000; Yarden and Sliwkowski, 2001). Initially isolated from the mouse submaxillary gland based on its ability to stimulate premature eye opening and tooth eruption in neonatal mice, EGF is a 53 amino-acid polypeptide derived by proteolysis from a larger precursor (Carpenter and Cohen, 1979; Cohen, 1986). The ability of EGF to stimulate growth and differentiation of epidermal and mesodermal tissues led both to its name and to keen interest in its mode of action.

Following its isolation, EGF was shown to bind with high affinity to a specific receptor in the cell membrane and stimulate rapid activation of a protein kinase activity (Carpenter *et al.*, 1975, 1978, 1979; Das *et al.*, 1977; Hock *et al.*, 1979; Wrann *et al.*, 1979). Purification and characterization of the EGF receptor (EGFR) showed it to be a ~170 kD molecular weight integral membrane glycoprotein (Cohen *et al.*, 1982). The ligand-inducible kinase activity co-purified with EGFR, suggesting a physical linkage between the ligand binding and kinase activities, which was later verified by molecular cloning (Cohen *et al.*, 1980; Ullrich *et al.*, 1984). Early on, the EGFR kinase activity was shown to result in phosphorylation of tyrosine residues, the first such demonstration for any receptor (Ushiro and Cohen, 1980).

ADVANCES IN PROTEIN CHEMISTRY, Vol. 68

0065-3233/04 $35.00

Molecular cloning of EGFR revealed it to be a 1186 amino acid protein with a 621 amino acid extracellular region followed by a single membrane-spanning region and a cytoplasmic tyrosine kinase (Ullrich *et al.*, 1984). Despite nonhomologous ligand binding regions, this overall architecture is shared by many other receptors, including those for insulin, PDGF, FGF, and VEGF, and these receptors are now collectively known as *receptor tyrosine kinases* (RTKs) (Schlessinger, 2000). The extracellular ligand-binding region of EGFR is made up of four subdomains arranged as a tandem repeat of two types of domains (Fig. 1). The first and third domains are homologous to one another and have been designated domains I and III or L1 and L2, respectively; the second and fourth domains are also homologous to one another and have been designated domains II and IV or CR1 and CR2, respectively (Bajaj *et al.*, 1987; Lax *et al.*, 1988; Ward *et al.*, 1995). The CR in this case is short for ''cysteine-rich'' and reflects the fact that nearly 50 conserved cysteines are found in these two domains. For simplicity, this review will employ the I, II, III, and IV domain nomenclature. Studies with mutant and chimeric EGF receptors and receptor fragments demonstrated that ligand binding is mediated primarily by domain III with some contribution from regions on domain I (Kohda *et al.*, 1993; Lax *et al.*, 1989). Curiously, the presence of domain IV was shown to be slightly inhibitory to ligand binding (Elleman *et al.*, 2001).

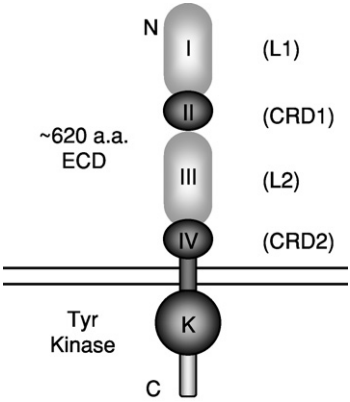


FIG. 1. Domain architecture of ErbB receptors. ErbB receptor extracellular regions are composed of four subdomains arranged as a tandem repeat of two types of domains. Two domain nomenclatures have been proposed (Bajaj *et al.*, 1987; Lax *et al.*, 1988; Ward *et al.*, 1995). The domains in order from the N-terminus are referred to as domain I (L1), II (CR1), III (L2), and IV (CR2). Domains I and III are homologous; domains III and IV are homologous. The extracellular region is followed by a single membrane-spanning region, a cytoplasmic tyrosine kinase, and variable length tail that harbors several phosphorylation sites.

Once the topology and functional organization of EGFR became apparent, questions about receptor activation focused on how extracellular ligand binding activates the intracellular kinase. Early studies with fluorescent-labeled EGF showed aggregation of EGFR on the cell surface in response to ligand binding (Schechter *et al.*, 1979), implicating receptor cross-linking as an activation mechanism. EGFR is endocytosed following ligand binding, however, and it was difficult to distinguish between aggregation as the trigger of signaling as opposed to a downstream response to receptor activation (Haigler *et al.*, 1979). A key piece of the puzzle emerged with the observation of ligand-induced dimers of EGFR (Yarden and Schlessinger, 1987), which was the first indication that dimerization might play a role in signaling for any receptor (Heldin, 1995). Ligand-induced dimerization—more broadly induction of a specific oligomeric conformation by ligand binding—is now accepted as the signaling trigger for all RTKs (Heldin, 1995; Schlessinger, 2000), as memorably illustrated by the crystal structure of the complex of human growth hormone with two of its receptors (de Vos *et al.*, 1992).

Prior to molecular cloning, amino-acid sequence data from EGFR revealed that the ErbB oncogene of the avian erythroblastosis virus encodes a truncated form of EGFR (Downward *et al.*, 1984). This truncated form is missing most of the extracellular region but includes a constitutively active kinase region that is responsible for unregulated growth of infected cells (Frykberg *et al.*, 1983; Yamamoto *et al.*, 1983). Demonstration that an oncogene encoded an activated form of a growth factor receptor provided exciting insight into the origins of cancer and presaged discovery of the involvement of EGFR and related receptors in the genesis and severity of many human cancers (Blume-Jensen and Hunter, 2001; Holbro *et al.*, 2003; Tang and Lippman, 1998). The nature of the ErbB oncogene also indicated that the extracellular region not only mediates ligand-dependent activation but also contributes to maintaining the kinase in an inactive state in the absence of ligand.

II. The EGF and EGFR Families

Both EGF and EGFR are archetypes of protein families that have undergone duplication and diversification throughout animal evolution (Muller and Schacke, 1996; Stein and Staros, 2000). *C. elegans* utilizes a single homolog of both EGFR (Let-23) and EGF (Lin-3), *Drosophila* utilizes a single EGFR (DER) and four EGF homologs (Vein, Spitz, Gurken, and Argos), and humans utilize four EGFR and at least 11 EGF homologs (Stein and Staros, 2000). The four human EGFR homologs are known as

both the HER (HER1, HER2, HER3, and HER4 for Human EGF Receptor) and ErbB (ErbB1, ErbB2, ErbB3, and ErbB4) families. EGFR is still commonly used to indicate HER1/ErbB1, and HER2 (ErbB2) is the cellular form of the *neu* oncogene product (Bargmann *et al.*, 1986b). This review will refer to the family of receptors as ErbB receptors and the individual receptors as EGFR, HER2, HER3, or HER4. The soluble extracellular regions of these receptors will be referred to as sEGFR, sHER2, sHER3, and sHER4.

EGF-related ligands include EGF, transforming growth factor-α (TGFα), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, betacellulin, and several isoforms of heregulin/neuregulin (Yarden and Sliwkowski, 2001). These ligands, which are derived by proteolysis from divergent precursors, are typically ~55 amino acids in length (Stein and Staros, 2000) and are characterized by a conserved pattern of 3 disulfide bonds and a loop-rich structure. EGF-related ligands may be categorized into the neuregulins, which primarily activate HER3 and/or HER4, and all others, which primarily activate EGFR (Fig. 2). Some cross-reaction between these ligand and receptor classes does occur, however. The sequences of EGF family ligands can be quite diverse; EGF and TGFα both bind and activate EGFR but share only 40% sequence identity.

The four human EGFR homologs share 40–45% sequence identity (Stein and Staros, 2000) but have become functionally specialized (Carpenter, 1987, 2003; Citri *et al.*, 2003; Olayioye *et al.*, 2000; Yarden and Sliwkowski, 2001). Each ErbB receptor has a ~220 amino-acid region following the kinase region that harbors multiple phosphorylation sites that become modified when the receptor is activated. Phosphorylation of the different sites recruits binding of different activators and initiates a characteristic pattern of downstream signaling events (Yarden and Sliwkowski, 2001). Expression patterns for each ErbB receptor and ligand are different and reflect involvement of each receptor in mediating growth and differentiation of diverse cell and tissue types (Olayioye *et al.*, 2000). Knockout of any of the ErbB receptors is embryonic lethal in the mouse with overlapping defects. Affected tissues include brain, skin, lung, and gastrointestinal tract (ErbB1) (Miettinen *et al.*, 1995; Sibilia and Wagner, 1995; Threadgill *et al.*, 1995), heart and brain (ErbB2 and ErbB3) (Lee *et al.*, 1995; Morris *et al.*, 1999; Riethmacher *et al.*, 1997), and heart (ErbB4) (Gassmann *et al.*, 1995).

Much of the phenotypic overlap observed in ErbB knockout mice appears to arise from the importance of heteromeric receptor combinations in mediating growth and development of specific tissues. In particular, HER2 and HER3 are unable to signal by themselves and must pair with another ErbB receptor to generate a signal. Despite much effort, no high-affinity

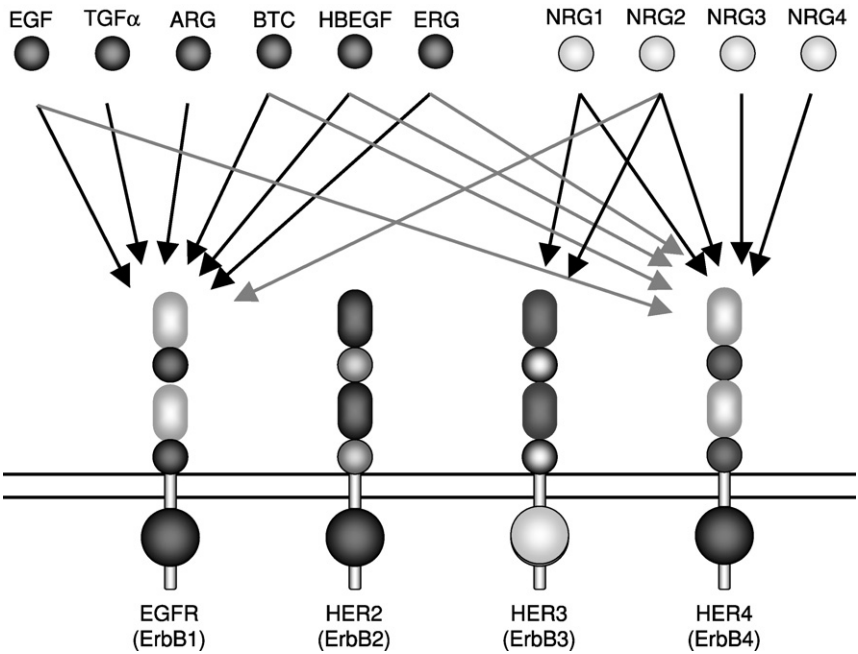


FIG. 2. Cognate ligand/receptor pairs. EGF-like ligands that activate ErbB receptors include Transforming Growth Factor α (TGFα), Amphiregulin (Arg), Betacellulin (Btc), Heparin-binding EGF (HBEGF), Epiregulin (Erg), and Neuregulin (Nrg, also known as Heregulin) (Yarden and Sliwkowski, 2001). Ligand-receptor interactions that induce strong responses are indicated by black arrows. Interactions that induce moderate responses are indicated by gray arrows (Klapper *et al.*, 2000).

ligand for HER2 has been identified, and all HER2-mediated signaling appears secondary to activation of other ErbB receptors (Klapper *et al.*, 1999). Indeed, all other ErbB receptors are able to form heteromeric signaling complexes with HER2 when co-expressed on the same cell. HER3, on the other hand, lacks an active kinase domain owing to mutation of several residues required for kinase activity (Guy *et al.*, 1994). Without an active kinase, HER3 must pair with and activate another ErbB receptor to generate a signal. The preferred partner of HER3—and indeed of EGFR and HER4—appears to be HER2, and the HER2/HER3 pair generates the strongest proliferative signal in many assays (Citri *et al.*, 2003). Because of the absence of a HER2 ligand and the inability of HER3 to activate an intrinsic kinase activity in response to ligand, these receptors have been referred to as the ''deaf and the dumb'' of the ErbB receptors (Citri *et al.*, 2003).

HER4 is unique among ErbB receptors in having been shown to undergo multiple proteolytic cleavages followed by translocation of its cytoplasmic region to the cell nucleus (Carpenter, 2003). The physiological significance of this processing is as yet poorly understood but raises interesting new possibilities for the generation and regulation of HER4-mediated signals.

III. Association of ErbB Receptors with Human Disease

EGFR became the first cell-surface receptor linked to cancer when Cohen and colleagues demonstrated downregulation of EGFR following transformation of cultured cells with specific oncogenic viruses (Todaro *et al.*, 1976). The significance of this association was not immediately clear, but discovery that the ErbB oncogene encoded an activated form of the EGFR kinase established a clear link between inappropriate EGFR activity and cancer (Downward *et al.*, 1984). In the 20 years since this discovery, dysregulated forms of ErbB receptors have been found to contribute to the genesis and severity of many human cancers (Blume-Jensen and Hunter, 2001; Holbro *et al.*, 2003; Tang and Lippman, 1998). ErbB dysregulation has been shown to occur through mutation (Humphrey *et al.*, 1990), overexpression of the receptor (Arteaga, 2002), or as a secondary effect downstream of inappropriate ligand expression (Sizeland and Burgess, 1992).

HER2 presents a particularly instructive example of growth factor receptor involvement in cancer. Molecular cloning of HER2 was nearly contemporaneous with its identification as the normal counterpart of the *neu* oncogene (Bargmann *et al.*, 1986b; Yamamoto *et al.*, 1986). The oncogene *neu* is responsible for the appearance of mutagen-induced neuroblastomas in rats and encodes a mutant form of the HER2 receptor, which frequently contains a single valine to glutamate change in the membrane-spanning region (Bargmann *et al.*, 1986a). This mutation appears to cause association and activation of the mutant receptors, which in turn leads to uncontrolled cell growth (Bargmann and Weinberg, 1988a,b).

Although the *neu* mutation has not been implicated in any human cancers, HER2 is overexpressed in 20–25% of human breast cancers, and this overexpression is correlated with more aggressive tumors and a significantly reduced survival time (Slamon *et al.*, 1987). This connection stimulated development of strategies targeting HER2 in HER2-overexpressing breast cancers, and a monoclonal antibody directed against HER2, Herceptin, has been approved for treatment of these cancers (Slamon *et al.*, 2001). Treatment with Herceptin has in some cases led to long-term remission, but the

mean improvement in life expectancy is currently 4–5 months (Slamon *et al.*, 2001). Earlier treatment with Herceptin (which has few side effects), combination of Herceptin with other therapies, or a better understanding of mechanisms of Herceptin resistance hold the promise of improved treatment strategies and outcomes, however.

IV. Structure of Individual ErbB Receptor Domains

Despite intense interest in ErbB receptors, high-resolution structural information has been slow in coming, owing largely to difficulties expressing and crystallizing these cysteine-rich glycoproteins. Fortunately, this situation has been remedied in the last year with publication of high-resolution crystal structures of active and inactive forms of the extracellular region of EGFR (sEGFR) (Ferguson *et al.*, 2003; Garrett *et al.*, 2002; Ogiso *et al.*, 2002), the extracellular region of HER2 (sHER2) both alone and complexed with the Herceptin Fab (Cho *et al.*, 2003; Garrett *et al.*, 2003), an inactive form of the extracellular region of HER3 (sHER3) (Cho and Leahy, 2002), and the EGFR kinase domain both alone and complexed with an inhibitor (Stamos *et al.*, 2002). These structures have yielded many unexpected but satisfying insights into the activation and regulation of ErbB receptors. At the same time, many new questions have been raised, and our appetite has been whetted for an even deeper molecular understanding of ErbB receptor function.

All structures of ErbB extracellular domains confirm the expected structural homology of the 'L' domains (domains I and III) to one another and to corresponding domains in type I insulin-like growth factor receptor (IGF1R). The structure of the first three domains of IGF1R, which are homologous to the first three domains of ErbB receptors, was determined in 1998 (Garrett *et al.*, 1998). Domains I and III share a beta-helical structure often referred to as a ''solenoid'' because it consists of 4–5 complete beta-helical turns containing a core of 22–24 amino acids per turn that stack to form an oblate cylinder (Fig. 3). The first beta-helical turn of domain I in the sEGFR/ligand complexes contains a 3-turn alpha helix that is adjacent to the bound ligand. The homologous region is disordered in the unliganded sHER3 structure, suggesting that interaction with ligand results in ordering of this helix in ErbB receptors.

A curious feature of domains I and III is a set of conserved asparagines—one per turn—with side chains that form hydrogen bonds to both main-chain atoms and the side chain atoms of the conserved asparagines of the next and preceding turns. These asparagines mediate a bend in the

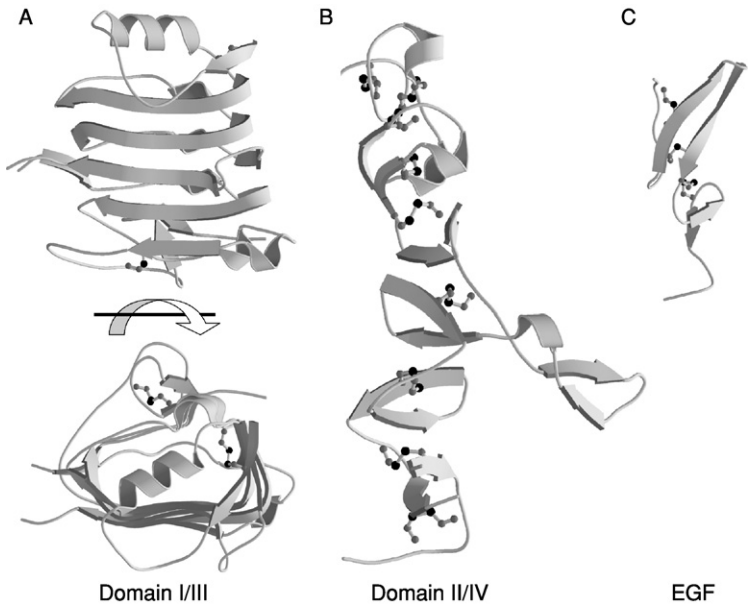


FIG. 3. Ribbon diagrams of ErbB receptor and ligand domains. (A) Orthogonal views of domain I from HER2 (Cho *et al.*, 2003). (B) Domain II from HER2 (Cho *et al.*, 2003). (C) EGF (Ogiso *et al.*, 2002). All ribbon diagrams in this and other figures were made using MOLSCRIPT (Kraulis, 1991).

main-chain within each turn, and the burial of their side-chains in the domain core appears to be accommodated by the inter-turn hydrogen bonds between the asparagine side chains of successive turns. These interactions result in an aligned ''line'' of buried asparagine side chains running along the beta-helical axis.

The cysteine-rich domains II and IV of ErbB receptors are homologous both to one another and to the cysteine-rich domain II of IGF1R (Burgess *et al.*, 2003; Garrett *et al.*, 1998). These domains can be characterized as a catenation of small disulfide bonded modules of ~15–20 amino acids that contain either a single disulfide bond that constrains a ''bow-like'' loop (C1 module) or two disulfide bonds in a Cys1-Cys3 and Cys2-Cys4 knot-like structure (C2 module). The pattern of modules in ErbB receptor domains II and IV are C2-C2-C2-C1-C1-C1-C1-C1 and C2-C1-C1-C2-C1-C1-C2, respectively (Ferguson *et al.*, 2003; Garrett *et al.*, 1998). Given the homology of the domain I/II and III/IV pairs, it seems likely that ErbB receptors arose as a duplication of an original domain I/II-like pair, suggesting that C1 and C2 modules are able to interconvert through mutation.

V. Structure of Entire ErbB Receptor Ectodomains

Although the structure of subdomains within ErbB receptor extracellular regions was anticipated from their homology to the IGF1R subdomains, their arrangement in intact receptors was not. The structures of unliganded HER3 and unactivated EGFR extracellular regions revealed a ~15 Å beta-hairpin loop that extends from domain II to interact with a pocket at the C-terminus of domain IV (Fig. 4). This interaction, akin to a ''snap'' or ''tether,'' constrains the EGFR and HER3 extracellular regions into a bracelet-like structure in which domains I and III are prevented from coming into close contact. The domain II/IV interaction is mediated by three sets of side-chain interactions including a tyrosine from domain II (Tyr 246 in HER3) that makes a hydrogen bond to a salt-bridged aspartate-lysine pair from domain IV (Lys 583 and Asp 562), a phenylalanine from domain II (Phe 251) that packs on a glycine from domain IV (Gly 563), and a glutamine from domain II (Gln 252) that makes a hydrogen bond to a histidine from domain IV (His 565) (Fig. 5). With the exception of Phe 251 and His 565, which are replaced by the similar residues tyrosine in EGFR and asparagine in HER4, respectively, each of these residues is conserved in all ErbB receptors except HER2.

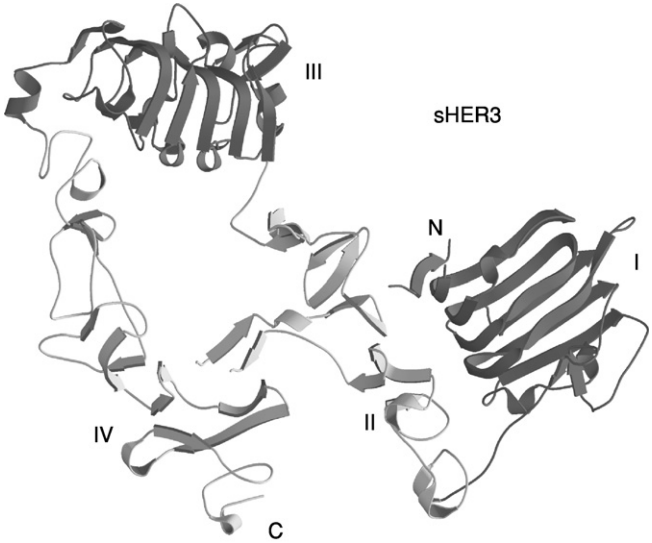


Fig. 4. Ribbon diagram of sHER3 (Cho and Leahy, 2002). Individual domains and the N- and C-termini are labeled. Domains I and III are colored dark gray; domains II and IV are colored light gray.

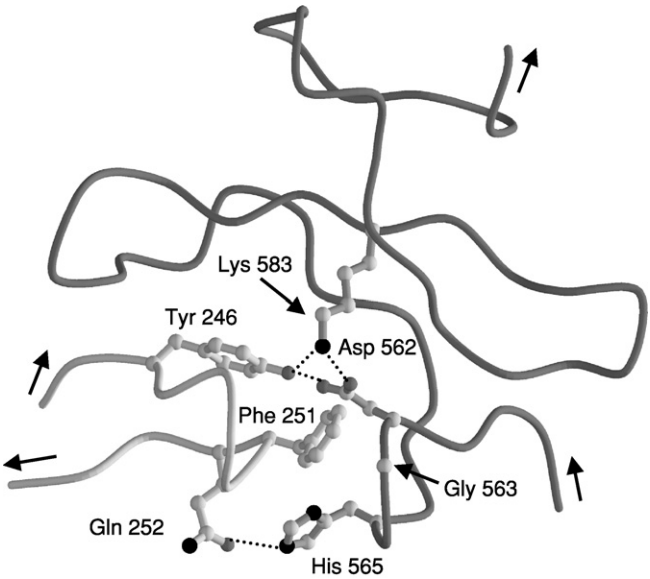


FIG. 5. Interactions in the HER3 domain II/IV ''snap'' contact region. Residues in the domain II loop (light gray) that contact residues in the domain IV pocket (dark gray) are indicated.

Structures of the first three subdomains of EGFR complexed with either EGF (Ogiso *et al.*, 2002) or TGFα (Garrett *et al.*, 2002) proved very similar to one another but revealed an entirely different arrangement of receptor subdomains than observed in unactivated forms of sEGFR and sHER3. Both EGF and TGFα bind to surfaces on domains I and III and mediate a close juxtaposition of these two domains (Fig. 6). The ''snap-like'' loop from domain II that mediates an interaction with domain IV in the unactivated state no longer contacts domain IV but instead mediates an inter-receptor dimer. The importance of the domain II loop for signaling has been demonstrated by site-directed mutagenesis (Garrett *et al.*, 2002; Ogiso *et al.*, 2002). In the case of the EGFR/EGF complex, domain IV was present in the crystal (although not visible in the electron density) indicating that participation of the domain II loop in mediating interreceptor dimers is favored over interactions with domain IV when ligand is present (Ogiso *et al.*, 2002).

A striking feature of ligand-induced dimers of sEGFR is that the dimer interface is mediated entirely by interreceptor contacts (Garrett *et al.*,

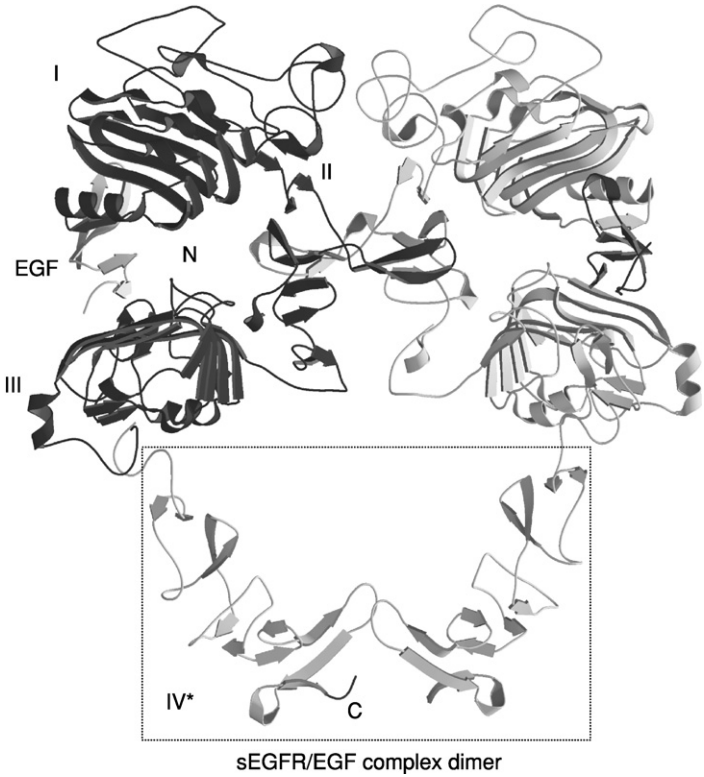


FIG. 6. Dimer of EGFR/EGF complex (Garrett *et al.*, 2002; Ogiso *et al.*, 2002). One EGFR subunit is colored dark gray and its bound EGF light gray; the other EGFR is colored light gray and its bound EGF dark gray. The relatively conserved interdomain orientation between domains III and IV of ErbB receptors has been used to model the position of domain IV, enclosed in a dashed box (Ferguson *et al.*, 2003).

2002; Ogiso *et al.*, 2002). In all previous structures of complexes of growth factor receptors with ligand, the ligand directly cross-links receptors (Schlessinger, 2000). An entirely receptor-mediated dimer allows formation of heteromeric receptor complexes without requiring ligand to bind simultaneously to two receptors. Co-receptors may thus evolve without requiring co-evolution of a ligand. Additionally, inter-receptor dimers mediated entirely by membrane-bound components may explain in part the weak association of ErbB extracellular regions that is observed in solution (Brown *et al.*, 1994; Lemmon *et al.*, 1997). The greatly increased local concentration that results from being confined to two dimensions

may not require as strong an association to drive dimerization as is needed when one or more components is free to move in three dimensions (Grasberger *et al.*, 1986).

Comparison of structures of different ErbB receptors indicates that both the domain I/II and domain III/IV pairs retain a relatively fixed interdomain orientation (Fig. 7). Some spine-like movement of the submodules of domain II is observed, but differences between ErbB structures appear to arise primarily from rigid body movements of the domain I/II and III/IV pairs relative to one another. The relatively fixed orientation between domains III and IV allowed modeling of the position of domain IV in the ligand-induced dimers of sEGFR (Burgess *et al.*, 2003) (Fig. 6), which showed that the C-termini of subunits within the dimer are located adjacent to one another. Close proximity of the juxtamembrane regions of subunits of a signaling dimer is required if receptor autophosphorylation occurs in trans as generally believed, and this result provides added confidence that the dimer observed in the sEGFR crystals is in fact a physiological dimer.

Receptor dimerization does not appear sufficient for signaling, however. Introduction of interreceptor disulfide bonds at different sites within the juxtamembrane region of ErbB receptors results in both active and inactive dimers (Burke *et al.*, 1997). Requirement for a stereospecific dimer is also seen for cytokine receptors (Jiang and Hunter, 1999) and suggests that conformational information is transmitted across the plasma

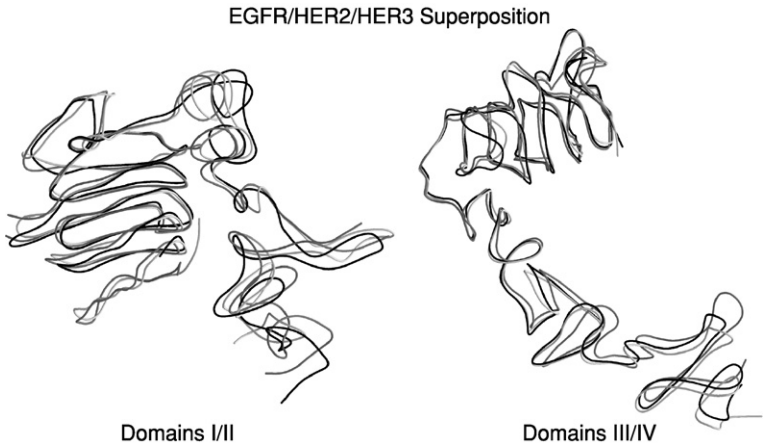


FIG. 7. Superposition of domain I/II and domain III/IV pairs from EGFR (black) (Ferguson *et al.*, 2003), HER2 (gray) (Cho *et al.*, 2003), and HER3 (white) (Cho and Leahy, 2002).

membrane during signaling. That is, simply inducing proximity of the cytoplasmic kinase regions is not sufficient to lead to activation.

A consequence of the recent structures of ErbB extracellular regions is the emergence of a simple model for ligand-induced dimerization of ErbB receptors (Burgess *et al.*, 2003) (Fig. 8). This model was as unexpected as it is satisfying in explaining several unusual features of ErbB receptors. In the unliganded state, the extracellular regions of EGFR, HER3, and—almost certainly—HER4 adopt a constrained structure in which an extended hairpin loop from domain II binds to a pocket at the C-terminus of domain IV. This conformation results in a large separation between domains I and III, which together comprise the ligand binding site. For ligand to bind, the domain II/IV connection must be broken and a large (~130°) rotation of the domain I/II pair relative to the domain III/IV pair must occur. This change brings together domains I and III to form a complete binding site and exposes the extended domain II loop, which is free to mediate inter-receptor dimerization and initiate signaling. The extended domain II loop

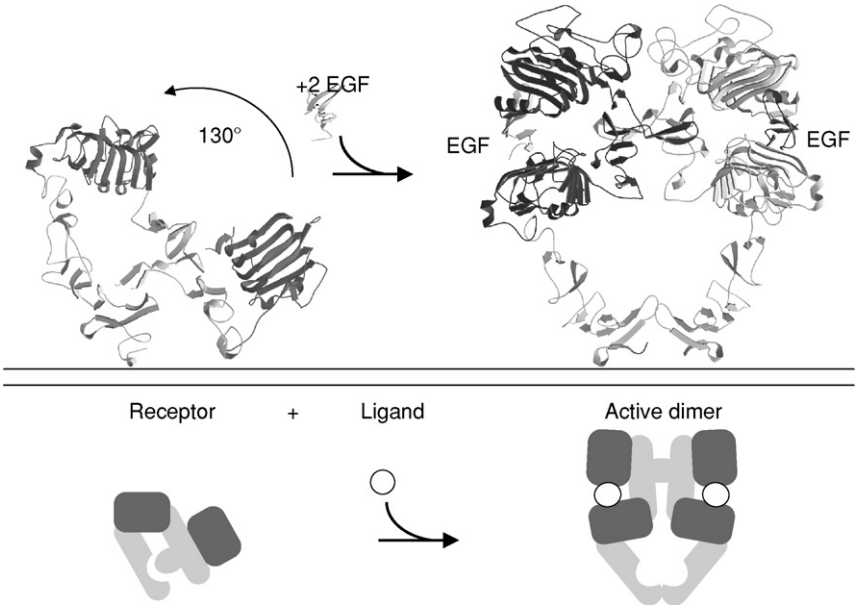
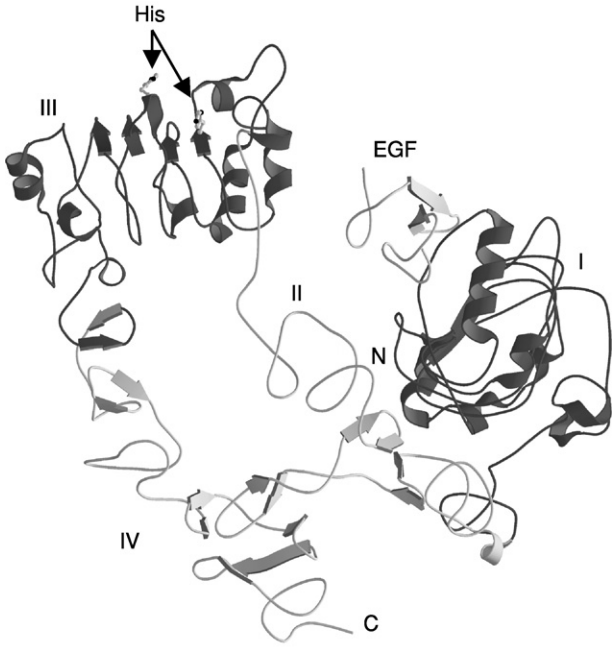


FIG. 8. Ligand-induced signaling mechanism. Structures and models of unliganded sHER3 (left) and ligand-bound sEGFR dimers (right) are shown. EGFR with bound ligand exhibits a ~130° rotation of the domain I/II pair relative to the domain III/IV pair when compared to unactivated sEGFR (Ferguson *et al.*, 2003) or sHER3 (Cho and Leahy, 2002). Adapted from a figure in Burgess *et al.* (2003).

has thus been called the ''dimerization'' loop. An essential feature of this model is a weak domain II/IV contact, which is consistent with the fact that its elimination by deletion or mutagenesis results in only a ~5–10 fold increase in the affinity of the receptor for ligand (Elleman *et al.*, 2001; Ferguson *et al.*, 2003).

The structure of a complex of the EGFR extracellular region and EGF determined at low pH provides a snapshot of a likely mechanism for release of bound ligand in the low pH environment of the endosome. EGFR is endocytosed after interacting with ligand, and at least some of this receptor is trafficked to endosomes where ligand dissociates. In crystals of a sEGFR/EGF complex grown at pH 5.0, EGF is bound only to the domain I binding surface, and the snap-like contact between domains II and IV is present (Ferguson *et al.*, 2003) (Fig. 9). Several histidines are conserved at the interface between EGF and domain III of EGFR, and protonation of



Low pH sEGFR/EGF complex

FIG. 9. Low pH form of an sEGFR/EGF complex (Ferguson *et al.*, 2003). Individual domains and the N- and C-termini are labeled; domains I and III are colored dark gray, and domains II and IV are colored light gray. EGF is colored light gray, and two histidines in domain III (His 346 and His 409) that contact EGF in the sEGFR/EGF complex formed at high pH (Garrett *et al.*, 2002; Ogiso *et al.*, 2002) are indicated.

these histidines at low pH appears to disrupt this interface (Ferguson *et al.*, 2003; Garrett *et al.*, 2002). Most of the interaction energy between EGFR and EGF is mediated by domain III (Kohda *et al.*, 1993). The interaction of EGF with domain I observed in these crystals is likely to be driven by the high concentrations of receptor and ligand used in crystallization trials. This structure thus appears to recapitulate events in the endosome—low pH results in protonation of histidines at the interface between EGF and domain III of EGFR leading to disruption of this interface and loss of high-affinity ligand binding.

VI. HER2

HER2 is unique among ErbB receptors in that no high-affinity HER2 ligand has been found, it functions as a co-receptor with each of the other ErbB receptors, and it is transforming when overexpressed (Di Fiore *et al.*, 1987; Klapper *et al.*, 1999). Much attention has been focused on HER2 because it is activated in many cancers (Holbro *et al.*, 2003; Slamon *et al.*, 1987; Tang and Lippman, 1998) and a HER2-targeted therapy, the monoclonal anti-HER2 antibody Herceptin, has demonstrated efficacy in a subset of breast cancers (Slamon *et al.*, 2001).

Structures of the entire extracellular region (Cho *et al.*, 2003) and the first three domains of HER2 (Garrett *et al.*, 2003) show it to adopt a very different conformation than unactivated forms of sEGFR or sHER3. The ''snap-like'' hairpin loop from domain II is present in HER2, but it is exposed to solvent and does not mediate a contact with domain IV (Fig. 10). Instead, an extensive ($\sim$1200 Å^2) and highly complementary contact is made between domains I and III, which appears to fix the orientation of the domain I/II pair relative to the domain III/IV pair. The interface between domains I and III is conserved in all three crystal forms of HER2 and appears to be a fixed feature of HER2 homologs. Two key hydrophobic residues buried at this interface, Leu 443 and Leu 472, are conserved in all HER2 homologs but not in other ErbB receptors. The HER2 domain I/II and III/IV pairs align well with the corresponding pairs from all other ErbB receptors (Fig. 7), suggesting that the interdomain orientations within these pairs are relatively rigid. By fixing the orientation of the domain I/II and III/IV pairs relative to one another, the HER2-specific interaction between domains I and III thus fixes the conformation of the entire HER2 extracellular region.

In light of the mechanism of ligand-induced signaling apparent from the sEGFR and sHER3 structures (Fig. 8), the structure of the HER2 extracellular domain explains several of its unique properties. The surface buried between domains I and III of HER2 overlaps significantly with