

Ligand-Induced, Receptor-Mediated Dimerization and Activation of EGF Receptor

Minireview

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The EGF receptor mediates many cellular responses in normal biological processes and in pathological states. Recent structural studies reveal the molecular basis for ligand binding specificity and how ligand binding induces receptor dimerization. Receptor dimerization is mediated by receptor-receptor interactions in which a loop protruding from neighboring receptors mediates receptor dimerization and activation.

Receptor and cytoplasmic protein tyrosine kinases play a prominent role in the control of a variety of cellular processes during embryonic development and in the regulation of many metabolic and physiological processes in a variety of tissues and organs (Hunter, 2000; Schlessinger, 2000; Pawson et al., 2001). Consequently, dysfunctions in the action of protein tyrosine kinases or aberrations in the activities and cellular localization of key components of the signaling pathways they activate will result in severe diseases such as cancer, diabetes, immune deficiencies, and cardiovascular diseases, among many others (Blume-Jensen and Hunter, 2001).

The epidermal growth factor receptor (EGFR) was the first receptor tyrosine kinase (RTK) to be discovered (Carpenter et al., 1978). Moreover, most of the principles and paradigms that underlie the action of receptor tyrosine kinases were first established for the EGFR (Schlessinger, 2000). Similarly, many of the mechanisms for activation and recruitment of intracellular signaling pathways following growth factor stimulation were discovered in studies of signaling via EGF receptors (Pawson and Schlessinger, 1993). Following the identification of EGFR, three additional members of the same receptor family were identified, ErbB2, ErbB3, and ErbB4. These receptors can be activated by EGF, TGF α , and ten additional potential ligands. Activating mutations and overexpression of members of this family of receptors were implicated in a variety of cancers, including mammary carcinomas, squamous carcinomas, and glioblastomas, as well as other malignant diseases (Blume-Jensen and Hunter, 2001).

Like all RTKs, the EGFR family is composed of an extracellular ligand binding domain, followed by a single transmembrane domain and a cytoplasmic domain containing a conserved protein tyrosine kinase (PTK) core, flanked by regulatory sequences. The extracellular domains of the EGFR family members are composed of four subdomains designated domain I, II, III, and IV or L1, S1, L2, and S2, respectively. It was proposed that domains I and III form the ligand binding domain of EGFR while other parts of the extracellular domain mediate receptor dimerization and interactions with other mem-

brane proteins (Lax et al., 1989). It is now well established that growth factor-induced receptor oligomerization is responsible for the activation of receptor tyrosine kinases and other receptors that contain a single transmembrane domain (reviewed in Jiang and Hunter, 1999). Growth factor-induced receptor dimerization is followed by intermolecular autophosphorylation of key tyrosine residues in the activation loop of the catalytic PTK domain resulting in stimulation of PTK activity. Tyrosine autophosphorylation sites in other parts of the cytoplasmic domain serve as docking sites for SH2 and PTB domains of signaling proteins that are recruited and activated upon growth factor stimulation (Pawson and Schlessinger, 1993).

While biophysical studies have shown that two EGF molecules bind to two receptor molecules within the context of EGFR dimers (Lemmon et al., 1997), the mechanism of EGF-induced EGFR dimerization was not understood. Now, two structures of ligand:receptor complexes are described in this issue of *Cell*. Garrett et al. (2002) describe the X-ray crystal structure of a deletion mutant of the extracellular domain of EGFR lacking almost the entire domain IV in complex with TGF α at 2.5 Å resolution. Ogiso et al. (2002) crystallized the entire extracellular domain of EGFR in complex with EGF and determined the structure at a resolution of 3.3 Å. Since most of domain IV is disordered, this structure contains the same region as seen in the deletion mutant lacking most of domain IV. Both structures provide a very similar view of a 2:2 receptor:ligand complex. Specifically, both structures demonstrate that TGF α or EGF make similar sets of contacts with a single EGFR molecule through specific regions in both domain I and domain III of the extracellular domain. The structure shows that the B loop of EGF interacts with domain I while residues from the A loop and from the C-terminal region of EGF interact with domain III of EGFR. Furthermore, the assignment of the EGF and TGF α binding domains in the two structures is consistent with previous mutagenesis and chemical crosslinking results. More than one dimeric arrangement of 1:1 ligand:receptor complex is observed in each structure due to crystal symmetry. In one dimer arrangement, domain I of one EGFR interacts with domain III of a second EGFR with a relative small buried surface area. The second dimer arrangement, termed the “back-to-back” dimer, is seen in both structures and is most likely to be physiologically relevant.

In this arrangement, the extracellular domain of EGFR dimerizes by virtue of homophilic interactions involving a specific loop projecting from each of the domain II cysteine-rich regions of the two adjoining extracellular domains (“dimerization loop”; Figure 1A). Indeed, point mutations in key residues or deletion of the dimerization loop prevents EGF-induced signaling via EGFR (Ogiso et al., 2002; Garrett et al., 2002)

Dimerization of EGFR requires the binding of two molecules of monomeric EGF to two EGFR molecules in a 2:2 EGF:EGFR complex formed from stable intermediates of 1:1 EGF:EGFR complexes (Lemmon et al., 1997).

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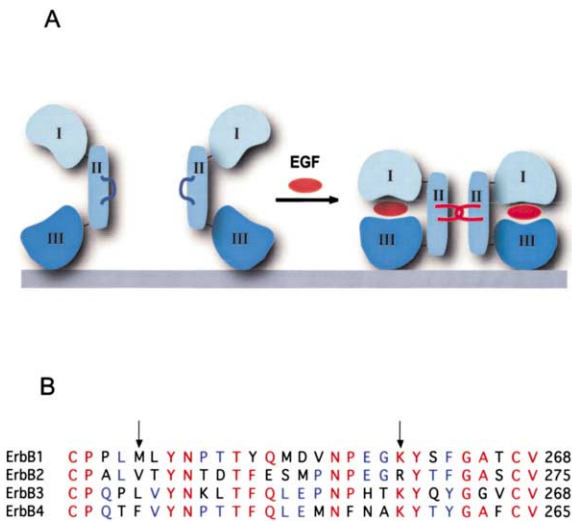


Figure 1. Model for Receptor-Mediated Mechanism of EGF Receptor Dimerization

(A) The crystal structures describe the ligand binding domain of EGF receptor in complex with EGF or TGF α . Receptor dimerization is mediated by a protrusion in domain II (dimerization loop, red color) that interacts with a specific region in the adjoining receptor to bring about receptor-receptor interactions. It is assumed that the dimerization loop in the unoccupied receptor adopts a conformation (colored blue) that does not facilitate receptor-receptor interactions. In addition, intramolecular domain II-IV interactions may also maintain the EGF receptor in an inactive state.

(B) Alignment of the amino acid sequence of the dimerization loop of EGF receptor (marked by arrows) with the corresponding sequences in ErbB2, ErbB3, and ErbB4. The residues in red and blue mark amino acids that are identical in three or two members of the EGF receptor family, respectively.

Two models were previously proposed for how EGF or Heregulin induce receptor dimerization. In one model, EGFR dimerization is mediated by the bivalency of EGF toward EGFR (Gullick, 1994), resulting in EGFR dimerization (“ligand-mediated” mechanism). This model is similar to the mechanism of growth hormone (GH)-induced dimerization of growth hormone receptor (GHR) in which a monomeric GH binds to two GHR molecules to form a 1:2 GH:GHR complex (de Vos et al., 1992). It was proposed that dimerization of EGFR and heterodimerization of erbB3 and erbB4 with other members of the EGFR family are mediated by the bivalency of EGF (Gullick, 1994) or Heregulin (Tzahar et al., 1997) toward EGFR and other members of the family. The crystal structures of EGFR in complex with EGF or TGF α described in the two papers appear to rule out this mechanism. In both structures, each ligand (EGF or TGF α) molecule is bound exclusively to a single EGFR molecule, and dimerization is mediated entirely by receptor-receptor interactions. The crystal structures are instead consistent with the “receptor-mediated” mechanism for dimerization (Lemmon et al., 1997), in which the binding of EGF to EGFR induces a conformational change that exposes a receptor-receptor interaction site in the extracellular domain, resulting in dimerization of two EGFR monomers only when occupied by EGF.

Regulation of Ligand Binding Affinity

Quantitative binding experiments demonstrated that EGF binds to EGFR with two distinct dissociation con-

stants: a minority (2%–5%) of high-affinity (0.1 nM) receptors and a majority (95%–98%) of low-affinity (10 nM) receptors. The high-affinity receptors are lost upon membrane solubilization or following protein kinase C-mediated phosphorylation of Thr-654 in the juxta-membrane domain of EGFR (reviewed in Ullrich and Schlessinger, 1990). In addition, a soluble form of the extracellular domain exhibits low-affinity binding toward EGF, while a truncated form lacking most of the second cysteine-rich domain (IV) binds EGF or TGF α with at least ten-fold higher affinity than the full-length ECD (Garrett et al., 2002). It was also reported that the binding affinity of EGF toward EGFR is modulated by coexpression of ErbB2, or ErbB3 in the same cells, two members of the receptor family that do not bind directly to EGF. Similar modulation of the binding affinity of Heregulin toward ErbB3 or ErbB4 was also detected in cells coexpressing EGFR or ErbB2. The crystal structures presented in the two reports provide new insights into these puzzling observations.

By comparing the previously described structure of the extracellular domain of the IGF1 receptor (Garrett et al., 1998) to the structure of the ligand-occupied EGFR, both groups propose that the conformation of the dimerization loop is coupled to the binding of EGF to the ligand binding cleft between domains I and III (Ogiso et al., 2002; Garrett et al., 2002). Moreover, comparison of the bound and unbound receptor structures suggests that the relative conformation and angle between domains I and III is altered as a consequence of ligand binding. This raises the possibility that the relative orientation of domains I and III is allosterically linked to a change in conformation of the dimerization loop that enables receptor dimerization. Moreover, changes in the conformation of the dimerization loop may influence the relative positions of domains I and III to control EGF binding affinity. Indeed, Garrett et al. (2002) demonstrate that deletion of the dimerization loop reduces ligand binding affinity and ligand-induced tyrosine autophosphorylation of the mutated EGFR. Bidirectional control, including “inside-out” regulation of EGF binding affinity toward EGFR that is induced by heterodimerization with other ErbB family members that do not bind EGF, could be mediated by their effects on the conformation of domain II and interactions mediated by the dimerization loop. In addition, because of the internal flexibility of the unoccupied extracellular domain of EGFR suggested by these structures, different populations of unbound EGFR may exist on the cell surface, exhibiting different orientations of domain I relative to domain III, possibly accounting for the heterogeneity in the dissociation constants of EGF toward EGFR. The recent structure of the extracellular domain of ErbB3 (Cho and Leahy, 2002) suggests that deletion of domain IV will release an autoinhibition conferred upon ligand binding by contacts between domains IV and II that constrain the relative orientation of domains I and III, resulting in reduced ligand binding affinity (Figure 2A).

Homo- and Heterodimerization of EGFR by a Receptor-Mediated Mechanism

EGFR is also known to undergo heterodimerization with ErbB2, ErbB3, or ErbB4 in response to EGF stimulation, resulting in their tyrosine phosphorylation (Graus-Porta et al., 1997). While ErbB3 and ErbB4 function as surface

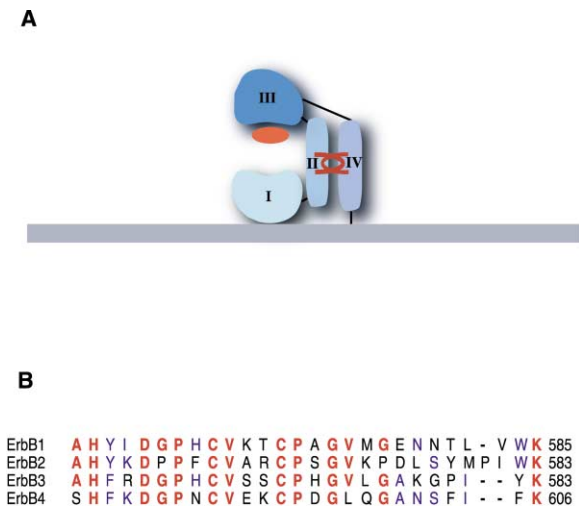


Figure 2. Autoinhibition of EGFR by Intramolecular Interactions
(A) A model for autoinhibition of EGFR based on the structure of ErbB3 (Cho and Leahy, 2002). Autoinhibition is mediated by domain II-IV contacts that constrain the relative orientation between domains I and III, resulting in reduced EGF binding affinity.
(B) Alignment of the amino acid sequence of a region in domain IV that is responsible for mediating domain II-IV interactions in ErbB3 and may play a similar role in other members of the EGFR family. This region may also participate in mediating additional receptor-receptor interactions. The residues in red and blue mark amino acids that are identical in three or two members of the EGFR family, respectively.

receptors for Heregulin, the physiological ligand of ErbB2 is still unknown. It is possible that ErbB2 may not have a ligand and that this receptor mediates its cellular responses by functioning as a partner for heterodimerization with EGFR, ErbB3, or ErbB4 (Graus-Porta et al., 1997). The receptor-mediated mechanism of EGFR dimerization provides a simple paradigm for homo- and heterodimerization of the EGFR family of receptors. Inspection of the primary structure of the dimerization loop shows that this loop is highly conserved among all members of the EGFR family (Figure 1B). It is plausible that the corresponding dimerization loops of ErbB3 or ErbB4 mediate Heregulin-induced homodimerization of the receptors. Moreover, the conservation of key residues of the dimerization loops of the different ErbB receptors suggests that this interaction may be sufficiently promiscuous to enable the formation of heterodimers between different members of the ErbB family of receptors. It was proposed that ErbB2 is a preferred partner for heterodimerization with all members of the EGFR family (Graus-Porta et al., 1997). A potential explanation for this phenomenon is that ErbB2 has evolved to act as an RTK without a ligand, with its dimerization loop maintained constitutively in the open, active configuration. This could allow homodimerization of ErbB2 in the absence of ligand and its heterologomerization with other members of the EGFR family following ligand-induced conformational change in their dimerization loops. Such a mechanism may account for the high activity of ErbB2 in human cancer cells that overexpress the ErbB2 protein. Because of the active configuration of its activation loop, dimerization may be driven by the

high concentration of ErbB2 even in the absence of ligand binding, resulting in stimulation of PTK activity and cell transformation.

What Is the Function of the Second Cysteine-Rich Domain?

The extracellular domain of all members of the EGFR family contains two homologous cysteine-rich domains designated domains II and IV or S1 and S2, respectively (Abe et al., 1998). The structures of the extracellular domain of EGFR described in the two papers do not provide any information about the second cysteine domain, since in one study most of this domain was deleted (Garrett et al., 2002) and in the second structure, domain IV was disordered (Ogiso et al., 2002). The recently described crystal structure of the extracellular domain of ErbB3 (Cho and Leahy, 2002) has shown that domain IV regulates ligand binding affinity by intramolecular interactions. Autoinhibition is mediated by interactions between domain IV and domain II that constrain the relative orientation of domains I and III, resulting in reduced ligand binding affinity (Figure 2A). The structure of EGFR shows that the first cysteine-rich domain is responsible for at least two functions; it acts as a scaffold that positions domains I and III in an orientation that generates a binding pocket for EGF and TGF α in the cleft formed between the two juxtaposed domains. In addition, the second cysteine-rich domain (domain IV) may interact with domain II via a conserved region that includes a cysteine-rich loop (amino acids 560–590) similar to the autoinhibitory intramolecular interactions revealed by the structure of the extracellular domain of ErbB3 (Figure 2B; Cho and Leahy, 2002). Following ligand binding and the release of the autoinhibition conferred by domain II-IV interactions, domain IV may function as a spacer that controls the orientation of the extracellular domain relative to the transmembrane domain and the plasma membrane. In addition, the putative dimerization loop in domain IV may function as an additional site of receptor-receptor interaction to stabilize the dimers formed by the dimerization loop of domain II and to mediate the formation of higher oligomerization states such as receptor tetramers.

Mechanism of Activation of EGFR

It is generally accepted that ligand-induced activation of receptor tyrosine kinases is mediated by intermolecular autophosphorylation of key tyrosine(s) residues in the activation loop of the catalytic PTK domain. In the inactive PTK, the activation loop adopts a configuration preventing access to ATP and substrate. Upon tyrosine phosphorylation, the activation loop adopts an “open configuration” enabling access to ATP and substrate, thus resulting in enhanced PTK activity. Indeed, mutations that render the activation loop in an open configuration result in enhanced PTK activity, while mutation of key tyrosine residues in the activation loop prevents PTK activation. By contrast to virtually all PTKs, mutation in a conserved tyrosine residue in the activation loop of EGFR is dispensable for protein tyrosine kinase activation and for signaling via the EGFR (Gotoh et al., 1992). This may indicate that the activation loop of EGFR does not play a prominent role in autoinhibition of the PTK domain of EGFR as shown for other protein tyrosine kinases. In other words, the inactive EGFR is not as tightly autoinhibited as the inactive forms of other PTKs.

Indeed, ErbB2 exhibits a PTK activity even in the absence of ligand stimulation. Moreover, the PTK activity is strongly enhanced upon overexpression of ErbB2 resulting in cell transformation.

The dimerization loop-mediated mechanism of receptor dimerization may function as a key regulatory step for control of the PTK activity of EGFR and other members of the family. Accordingly, EGF-induced receptor-mediated dimerization will facilitate intermolecular autophosphorylation that in turn will maintain the PTK domain in an active state. Receptor-mediated dimerization may also provide a potential explanation for the strong stimulation of the PTK activity caused by overexpression of erbB2. As the dimerization loop of ErbB2 is maintained in the active configuration, overexpression of ErbB2 will facilitate spontaneous dimerization of the receptor, resulting in *trans* autophosphorylation and further stimulation of ErbB2 PTK activity.

Future structural and biochemical experiments will reveal the potential role of the second cysteine-rich domain (domain IV) in receptor autoinhibition and control of receptor oligomerization and the important unanswered question of how ligand-induced receptor dimerization activates the cytoplasmic protein tyrosine kinase activity.

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