Conformational changes in receptor tyrosine kinase signaling: an ErbB garden of delights
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Abstract
The ErbB family of receptor tyrosine kinases plays important roles in cell proliferation, differentiation, and apoptosis. Recent structural studies of these receptors have demonstrated dramatic conformational effects that are critical to their ligand binding and activation, and have shown that these receptors provide levels of control beyond the classic dimerization/activation mechanism. These results indicate that this class of receptors has evolved subtle regulatory mechanisms via genetic and protein structural changes to influence their effects on cell behaviors.

Introduction and context
Receptor tyrosine kinases pass information from the exterior to the interior of cells. Binding of a ligand to an extracellular domain of the receptor triggers activation of the kinase domain at the cytoplasmic surface of the plasma membrane, resulting in phosphorylation of tyrosine residues of the receptor’s cytoplasmic sequences [1]. The classic mechanism for this transition is the dimerization of receptor molecules induced by ligand binding, which juxtaposes cytoplasmic domains to facilitate a transphosphorylation by the kinase domains that control cellular behaviors [3]. The prototype for development of this mechanism has been the epidermal growth factor (EGF) receptor and its family members, known as ErbBs. However, recent studies of this family have shown that their signaling mechanisms are much more complex and subtle than a simple dimerization model.

The ErbB family consists of four members that share a common domain structure consisting of four extracellular domains, a transmembrane domain, a juxtamembrane cytoplasmic domain, a kinase domain, and a C-terminal tail, which contains most of the phosphorylated tyrosines [3] (Figure 1). Although family members ErbB1 and ErbB4 can be phosphorylated directly after ligand binding and homodimerization, ErbB2 and ErbB3 cannot [4]. No soluble high-affinity ligand has been demonstrated for ErbB2 [4], and ErbB3 has an inactive kinase domain [5]. Thus, ErbB2 has been proposed to act primarily as a co-receptor through heterodimerization with the other three receptors and activation via their ligands [6]. In contrast, ErbB3 serves as a docking protein that is phosphorylated by the other family members. The ErbB2/ErbB3 pair is particularly potent for activating proliferation responses [7].

Major recent advances
A role for conformational effects in ligand binding to the ErbBs has been shown by crystallographic studies of their extracellular domain structures [8]. ErbB1, ErbB3, and ErbB4 were demonstrated to be in an intramolecular ‘tethered’ conformation in the absence of ligand, in which extracellular domains 2 and 4 are linked [9-11] (Figure 1). In contrast, these receptors in the presence of ligand are in an ‘extended’ conformation in which a loop in domain 2 is freed and serves as a coupling site for the
association of two receptor molecules in a dimer [12] (Figure 1). ErbB2 exhibits a different structure, as it is in an extended conformation in the absence of ligand, with its domain 2 loop available for interaction with the other ErbBs [13]. These crystallographic studies are supported by small x-ray scattering studies of the extracellular regions of ErbB1, ErbB2, and ErbB3 [14]. Since the ligand-binding site of the ErbBs is closed in the extended conformation, these results can explain the failure to find a soluble ErbB2 ligand. However, it should be noted that the ErbBs must exhibit considerable conformational fluctuations and flexibility as receptor dimers, such as the ErbB2/ErbB3 couple, can be formed even in the absence of ligand, although ligand is required for phosphorylation [15].

The mechanism of phosphorylation has been investigated by crystallographic studies and mutational analyses of the cytoplasmic domains of the EGF receptor. Surprisingly, these results showed an asymmetric interaction between the two kinase domains of the coupled receptors, in which the large lobe of one kinase domain (donor) associates with the small lobe of the other receptor (acceptor) (Figure 1), thus stabilizing the active conformation of the small lobe [16]. This activation mechanism resembles that of cyclin-dependent kinases, applies to both homodimers and heterodimers of ErbBs, and differs from that of most other receptor tyrosine kinases, such as the insulin receptor [16,17]. The ability of the ErbB kinase domains to serve as both activators and transducers of the ligand signal provides a powerful discriminatory mechanism for the regulation of signaling through these receptors.

Despite extensive studies, the exact relationship between EGF binding and EGF receptor dimerization has remained elusive. Recent work has shown that binding is positively linked to dimerization in unphosphorylated receptors but that the linkage is lost with receptor autophosphorylation [18]. In addition, these studies...
showed that ligand-binding affinity obeys negative cooperativity, in which ligand binding to the first subunit of the dimer decreases the affinity with which ligand binds to the second subunit in the dimer [19]. Significantly, this cooperativity is dependent on the presence of the intracellular juxtamembrane domain of the receptor [18]. These findings indicate that this domain can influence ligand binding and cause a type of inside-out signaling, providing further evidence that the ErbBs have evolved very subtle and sophisticated regulatory mechanisms for coupling ligand binding and phosphorylation.

Importantly, the juxtamembrane domain of the EGF receptor has also been shown to be involved in receptor activation [20,21]. Crystal structures of EGF receptor dimers that include the juxtamembrane region show that the C-terminal half of the juxtamembrane domain (JM-B) ‘latches’ the C-lobe of the donor kinase domain to the N-lobe of the acceptor domain (Figure 1), which stabilizes the dimer and promotes the allosteric activation of the acceptor tyrosine kinase domain. In the symmetric dimer, which is inactive, this interaction is prevented by the cytoplasmic tails of the receptors. Ligand engagement by the extracellular domains stabilizes the formation of the juxtamembrane domain interaction with the kinase domain, which in turn stabilizes the kinase domain dimer, a type of outside-in signaling.

**Future directions**

Although the conformational changes and preferences described here apply specifically to the ErbBs, it will be interesting to find out whether they are also exhibited in other receptors. Moreover, it remains to be seen how these conformational effects alter specific downstream signaling pathways. A continuing question for ErbB phosphorylation is how the activated kinase domain interacts with and phosphorylates particular residues of the cytoplasmic tail region to specify different pathways. One puzzle remaining for the ErbB2-ErbB3 heterodimer shown in Figure 1 is how the ErbB2 is phosphorylated since ErbB3 is ‘kinase dead’. Is this due to a ‘true autophosphorylation’ or to the formation of higher-order multimers, or to some other mechanism yet undiscovered? Finally, it is clear from the combined results of these efforts that the receptor molecules must be considered as a complete dynamic package, in which all of the domains, including the transmembrane domain, may contribute to their functions.

**Abbreviation**

EGF, epidermal growth factor.

**Competing interests**

The authors declare that they have no competing interests.

**Acknowledgments**

Original research cited herein was funded by National Institutes of Health (NIH) grants CA52498 and CA74072.

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