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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

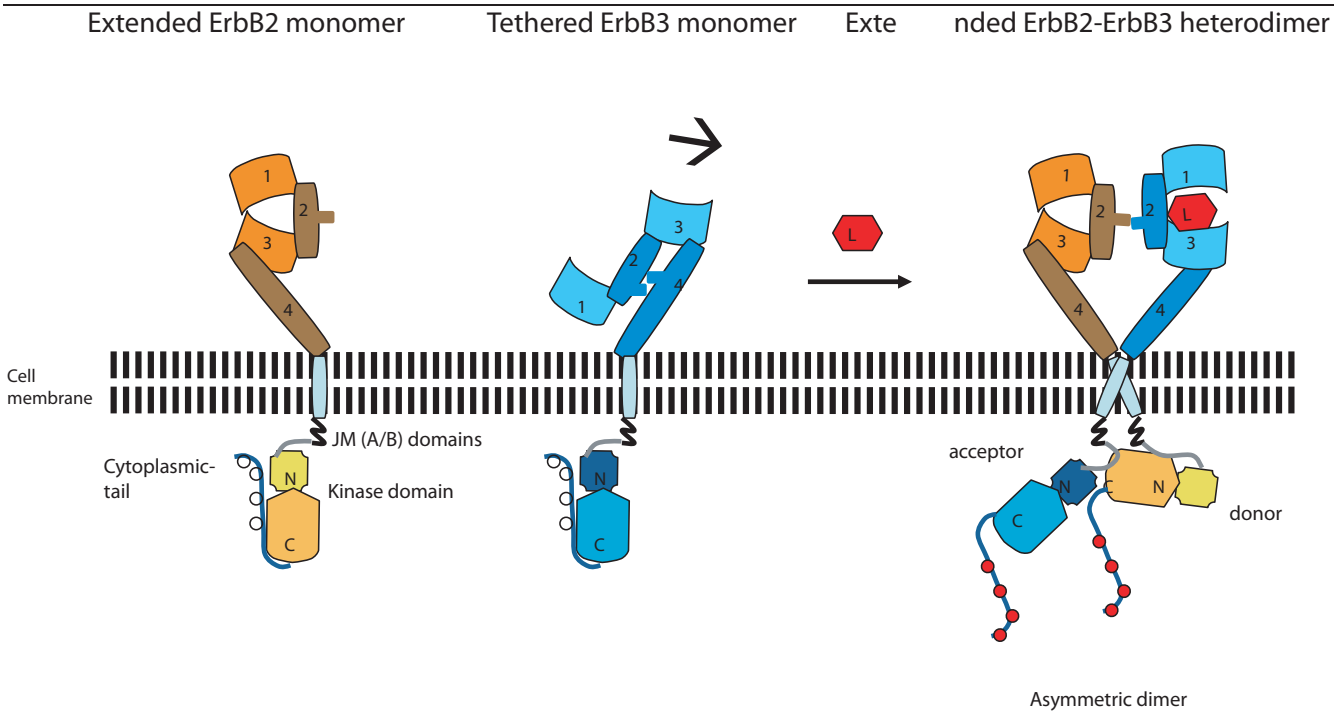
Receptor tyrosine kinases pass information from the exterior to the interior of cells. Binding of a ligand to an extracellular domain of the receptor triggers activation of the kinase domain at the cytoplasmic surface of the plasma membrane, resulting in phosphorylation of tyrosine residues of the receptor's 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 between the adjacent receptors [2]. The phosphorylated tyrosines then act to recruit cytoplasmic proteins, such as adaptors, docking proteins, and enzymes, which initiate downstream signaling pathways 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

Figure 1. A general model for the activation of members of the ErbB family, illustrating the case of the ErbB2-ErbB3 heterodimer



ErbB monomer conformation is depicted for the unique ErbB2 extended conformation and for the tethered ErbB3 monomer, also representing the conformation of ErbB1 and ErbB4. Ligand (L) is labeled in red, and the curved arrow provides a visual aid for the direction of conformational change from the tethered to the extended form in the presence of ligand. Extracellular domains are colored in shades of orange (ErbB2) and blue (ErbB3) and are numbered for further aid in visualizing the conformational changes. The transmembrane domain is in pale blue. Intracellular subdomains are labeled, and within the cytoplasmic tail, empty circles represent nonphosphorylated tyrosine residues and red circles represent tyrosine phosphorylation. An active ErbB2-ErbB3 heterodimer is also depicted and portrays the alignment of the extracellular dimerization domain, the asymmetric orientation of the kinase domains, and the role of the juxtamembrane region in promoting this orientation. How ErbB2 becomes phosphorylated in this specific dimer is not clear at this time.

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.

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References

1. Hubbard SR, Till JH: **Protein tyrosine kinase structure and function.** *Annu Rev Biochem* 2000, **69:**373-98.
2. Schlessinger J: **Ligand-induced, receptor-mediated dimerization and activation of EGF receptor.** *Cell* 2002, **110:**669-72.
3. Yarden Y, Sliwkowski MX: **Untangling the ErbB signalling network.** *Nat Rev Mol Cell Biol* 2001, **2:**127-37.
4. Klapper LN, Kirschbaum MH, Sela M, Yarden Y: **Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors.** *Adv Cancer Res* 2000, **77:**25-79.
5. Guy PM, Platko JV, Cantley LC, Cerione RA, Carraway KL 3rd: **Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity.** *Proc Natl Acad Sci U S A* 1994, **91:**8132-6.
6. Olayioye MA, Neve RM, Lane HA, Hynes NE: **The ErbB signaling network: receptor heterodimerization in development and cancer.** *EMBO J* 2000, **19:**3159-67.
7. Riese DJ 2nd, Stern DF: **Specificity within the EGF family/ErbB receptor family signaling network.** *Bioessays* 1998, **20:**41-8.
8. Lemmon MA: **Ligand-induced ErbB receptor dimerization.** *Exp Cell Res* 2009, **315:**638-48.
9. Burgess AW, Cho HS, Eigenbrot C, Ferguson KM, Garrett TP, Leahy DJ, Lemmon MA, Sliwkowski MX, Ward CW, Yokoyama S: **An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors.** *Mol Cell* 2003, **12:**541-52.
10. Cho HS, Leahy DJ: **Structure of the extracellular region of HER3 reveals an interdomain tether.** *Science* 2002, **297:**1330-3.

    F1000 Factor 6.7 *Must Read*
    Evaluated by Kristina Downing 14 Aug 2002, Mario Amzel 03 Sep 2002, Jia-huai Wang 04 Sep 2002, Kermit Carraway 05 Sep 2002
11. Bouyain S, Longo PA, Li S, Ferguson KM, Leahy DJ: **The extracellular region of ErbB4 adopts a tethered conformation in the absence of ligand.** *Proc Natl Acad Sci U S A* 2005, **102:**15024-9.
12. Ferguson KM: **Structure-based view of epidermal growth factor receptor regulation.** *Annu Rev Biophys* 2008, **37:**353-73.
13. Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, Kofler M, Jorissen RN, Nice EC, Burgess AW, Ward CW: **The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors.** *Mol Cell* 2003, **11:**495-505.
14. Dawson JP, Bu Z, Lemmon MA: **Ligand-induced structural transitions in ErbB receptor extracellular domains.** *Structure* 2007, **15:**942-54.
15. Tao RH, Maruyama IN: **All EGF(ErbB) receptors have preformed homo- and heterodimeric structures in living cells.** *J Cell Sci* 2008, **121:**3207-17.
16. Zhang X, Gureasko J, Shen K, Cole PA, Kuriyan J: **An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor.** *Cell* 2006, **125:**1137-49.

    F1000 Factor 9.3 *Exceptional*
    Evaluated by Giulio Superti-Furga 20 Jun 2006, Stevan Hubbard 23 Jun 2006, Gourisankar Ghosh 27 Jun 2006, Xiayang Qiu 27 Jun 2006, Karen Anderson 28 Jun 2006, Elizabeth Goldsmith 30 Jun 2006, Friedemann Kiefer 06 Jul 2006, Jane Endicott 31 Aug 2006, Frank Sicheri 12 Dec 2007
17. Kuriyan J, Eisenberg D: **The origin of protein interactions and allostery in colocalization.** *Nature* 2007, **450:**983-90.
18. Macdonald-Obermann JL, Pike LJ: **The intracellular juxtamembrane domain of the epidermal growth factor (EGF) receptor**

is responsible for the allosteric regulation of EGF binding. *J Biol Chem* 2009, **284:**13570-6.

F1000 Factor 8.0 *Exceptional*
Evaluated by Kermit Carraway 27 Apr 2009, Ralf Jockers 15 May 2009

19. Macdonald JL, Pike LJ: **Heterogeneity in EGF-binding affinities arises from negative cooperativity in an aggregating system.** *Proc Natl Acad Sci U S A* 2008, **105:**112-7.

F1000 Factor 3.0 *Recommended*
Evaluated by Stuart S Licht 18 Aug 2008

20. Red Brewer M, Choi SH, Alvarado D, Moravcevic K, Pozzi A, Lemmon MA, Carpenter G: **The juxtamembrane region of the EGF receptor functions as an activation domain.** *Mol Cell* 2009, **34:**641-51.

F1000 Factor 3.0 *Recommended*
Evaluated by Angel Nebreda 22 Jul 2009

21. Jura N, Endres NF, Engel K, Deindl S, Das R, Lamers MH, Wemmer DE, Zhang X, Kuriyan J: **Mechanism for activation of the EGF receptor catalytic domain by the juxtamembrane segment.** *Cell* 2009, **137:**1293-307.

F1000 Factor 5.1 *Must Read*
Evaluated by Matthias Buck 20 Jul 2009, Misha Sherman 21 Jul 2009, Angel Nebreda 22 Jul 2009, Stevan Hubbard 04 Aug 2009, John Ladbury 21 Aug 2009