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Juxtamembrane contribution to transmembrane signaling

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Summary

Signaling across the cell membrane mediated by transmembrane receptors plays an important role in diverse biological processes. Recent studies have indicated that, in a number of single-span transmembrane receptors, the intracellular juxtamembrane (JM) sequence linking the transmembrane helix with the rest of the cytoplasmic domain participates directly in the signaling process via several novel mechanisms. This review briefly highlights several modes of JM dynamics in the context of signal transduction that are shared by different types of transmembrane receptors.

Introduction

Transmembrane receptors play important roles in diverse biological processes. The molecular mechanism by which the receptors transmit the ligand-binding signals across the membrane bilayer is an important topic that has been under scrutiny for the past several decades. For many receptors containing a single-span transmembrane (TM) helix, such as receptor tyrosine kinases, cytokine receptors, immune receptors and cell adhesion receptors, the majority of the attention has been on the extracellular ligand-binding domains of a receptor as well as its interaction with the ligands. For the last decade or so, however, it has become evident that the TM helix in many of these receptors participates actively in the signaling process.

In comparison to the diverse range of ligand-induced conformational changes in the extracellular domain of membrane receptors, the planar nature of the cell membrane dictates that possible motions a TM helix can undertake to transmit signals across the membrane be limited to 4 types – translation (lateral translation in the membrane), piston (translation perpendicular to the membrane), pivot (rotation parallel to the membrane) and rotation (rotation perpendicular to the membrane)^{1, 2}. The translation motion as a result of association or dissociation of the TM helices is probably the most prevalent type and has received the most attention. In contrast, since the piston motion likely entails the exposure of a portion of the hydrophobic TM helix to the aqueous environment and therefore requires the input of free energy more significant than the other types, its occurrence is much rarer than the other types and therefore should be interpreted with caution^{3, 4}. A piston-like movement has been described in the bacterial aspartate receptor, as one TM helix moves, in

response to aspartate binding to the receptor, by approximately 1 Å relative to the other helix in the same subunit^{5, 6}.

In many cases the movement of TM helices during signal transmission involves a combination of 2 or more types. Regardless of the types of movement, during signal transmission the TM movement relative to the cell membrane is of a limited scale, often within a few angstroms. Thus, how such a slight movement causes a significant change in the post-translational modification of the receptor or in the association/dissociation with downstream effector proteins has increasingly become a topic of interest. A number of recent studies have suggested that the intracellular juxtamembrane (JM) sequence linking the TM helix with the rest of the cytoplasmic domain of the membrane receptor also participates directly in the signaling process. It appears to relay and amplify the signal from the TM helices into something more substantial or more recognizable. This brief review seeks to highlight some recent advances in the studies of JM sequence in the context of signal transduction.

Receptor tyrosine kinases (RTKs)

In the classical model of RTK signaling, the ligand binding induces conformational changes and dimerization of the receptor, and subsequently trans-autophosphorylation and activation of the kinase domain located in the intracellular domain⁷⁻⁹. This model is still valid, although it has been updated with details of conformational changes in each domain and variations observed in different subtypes of RTKs¹⁰. Recent studies have suggested that, in addition to helping to maintain the inactive conformation of the kinase domain¹¹, the intracellular JM region in the RTK can undergo a conformational change in tandem with the adjoining TM helix during dimerization of the receptor.

The canonical example of RTK signaling is the epidermal growth factor receptor (EGFR) and its related ErbB family members. Binding of EGF induces a conformational change in the extracellular domain of EGFR that facilitates its dimerization¹². Relatedly, a point mutation in the TM domain of ErbB2/Neu receptor was found to induce receptor dimerization and activation¹³⁻¹⁵. Recent biophysical characterization and comparison of TM-JM fragments in phospholipid vesicles that were derived from the wild-type or constitutively active mutant ErbB2/Neu receptors indicated that the JM region in the wild-type fragment was unstructured but bound to the negatively charged PIP₂-containing membrane¹⁶. It is likely that the interaction with the anionic lipids is mediated by the basic residues that are enriched in the JM sequence of ErbB2/Neu receptor¹⁷. Furthermore, the binding affinity of the JM sequence for the membrane was dependent on the relative orientation of the TM helix. In contrast, the JM region in the mutant TM-JM fragment was not bound to the membrane¹⁶. Consistently, structural analysis of the isolated TM-JM fragment derived from wild-type EGFR in phosphocholine bicelles and related functional analysis in the context of full-length EGFR suggested that the JM region forms an antiparallel coiled-coil homodimer upon formation of the activating TM helical dimer in the membrane^{18, 19} (Fig. 1A). The interface in the right-handed TM helical dimer of EGFR is located in the N-terminal portion of the TM domain, which leaves the C-termini of TM helices apart and positioned to accommodate the antiparallel JM dimer²⁰. Thus, from these

studies has emerged a model of EGFR signaling: the JM sequence is attached to the inner membrane surface in the inactive state. Upon ligand binding, the TM-JM domains form a dimer in which the JM sequence becomes detached from the membrane and facilitates formation of the asymmetric dimer of the kinase domains in which phosphorylation occurs (Fig. 1A).

The mode of involvement of the JM sequence in the signaling EGFR and its family member may be applicable to other similarly built RTKs, such as fibroblast growth factor receptor 3 (FGFR3)²¹. Some RTKs form dimers in the absence of a ligand. Even EGFR can form ligand-free dimer, the extent of which is related to its expression level in the plasma membrane^{22, 23}. Whereas the TM domain, but not the cytoplasmic domain, of discoidin domain receptor 1 (DDR1) is capable of mediating ligand-free dimerization²⁴, the JM sequence in closely related DDR2 contributes to the receptor dimerization and is essential to its activation²⁵. It remains unclear whether the JM sequence of DDR, which is substantially longer than that of other RTKs, cooperates with the TM domain in the dimerization process.

Cytokine receptors

Type I and II cytokine receptors differ from RTKs in that their cytoplasmic domain does not contain a kinase domain. Instead it relies on the activity of an associated kinase protein, Janus kinase (JAK), to activate downstream proteins in their signaling pathways. Unlike RTKs, many cytokine receptors are constitutive dimers on the cell surface, in which the TM domain has been shown to mediate the homodimerization²⁶⁻²⁹. Consequently the receptor activation should entail a ligand-induced conformational change in the extracellular domains within the dimeric receptor that couples with a change in the relative position between the two cognate JAKs. A recent study of growth hormone receptor (GHR) suggested that binding of growth hormone induces a closeup and rotation of the extracellular half of the TM helical dimer and concurrent separation of the intracellular half of the TM sequence³⁰ (Fig. 1B). The separation further induces activation of the cognate JAK2.

The conserved JM sequences on both sides of the TM helix can modulate the activation of the host receptor. Mutations in the extracellular JM region immediately preceding the TM helix that facilitate the close association activate GHR, providing support for the aforementioned model³⁰. Similarly, eltrombopag, a small molecule that activates thrombopoietin receptor (TpoR) to stimulate megakaryopoiesis and platelet production³¹, binds the extracellular JM and TM domain of TpoR by inducing presumably the self-association and/or rotation of this region^{32, 33}. Crosslinking of the extracellular JM-TM regions of erythropoietin receptor (EpoR) by engineered cysteine residues, but only at specific positions that likely represent one side of a helix, activates signal transduction pathways in the absence of the ligand³⁴. On the other hand, the intracellular JM region immediately following the TM domain of TpoR takes on a helical conformation, interacts with the membrane surface, and is important in keeping the receptor in an inactive state³⁵. In particular, mutations of residue Trp515 at the TM-intracellular JM junction that cause constitutive activation of TpoR alter the receptor dimerization state and also the tilting angle of the TM helix³⁶. Similarly, mutations in the intracellular JM region of GHR and EpoR have been reported to increase activation^{30, 37}.

Immunoreceptors

Many receptor complexes employed by the immune system, such as T cell receptor (TCR) and Fc receptors, are devoid of an intrinsic kinase domain or a cognate kinase protein. Instead, their cytoplasmic domains often are decorated with tyrosine-containing sequence motifs that, upon phosphorylation, attract the association of intracellular signaling proteins to propagate downstream signaling pathways. Upon ligand binding, these immunoreceptors often form clusters and partition into the detergent-resistant lipid rafts that are enriched with various kinases, which in turn phosphorylate the juxtaposed tyrosines located in the cytoplasmic domains of immunoreceptors³⁸⁻⁴⁰.

In many immunoreceptors, an immunoreceptor tyrosine-based activation motif (ITAM) is located adjacent to a JM sequence enriched with basic residues. Although with little discernible amount of stable secondary structure, the basic-rich JM sequence of CD3 ϵ binds to the plasma membrane, which facilitates burial of the nearby tyrosine into the membrane interior⁴¹ (Fig. 1C). Its dissociation from the negatively charged membrane is required for phosphorylation thereof, suggesting that the membrane association limits its access to membrane-bound kinases such as Lck and prevents its phosphorylation⁴¹ (Fig. 1C). Similarly, the basic-rich JM sequence within the cytoplasmic domain of CD3 ζ mediates association with the plasma membrane, and the ligand engagement with TCR results in its dissociation from the membrane^{42, 43}. Mutations of the CD3 ζ JM sequence that disrupt its high-affinity association with the phosphoinositide-containing membrane attenuate the responses induced by TCR engagement and also alter the co-localization of TCR complex with Lck⁴⁴, thus highlighting the functional importance of the JM sequence. Relatedly, it was reported recently that, following the initial TCR triggering, an increase in the intracellular calcium level interferes with the membrane-JM interaction and therefore induces dissociation of tyrosines from the membrane bilayer, thus making them accessible for phosphorylation by Lck⁴⁵. It should be noted that many of these JM sequences are intrinsically disordered despite their lipid-binding activity⁴⁶. The ability of basic-rich sequence to bind negatively charged plasma membrane, especially phosphoinositide-enriched membrane surface, is also noted in many other proteins⁴⁷⁻⁴⁹. Overall, these recent reports have suggested a novel mechanism by which the basic-rich JM sequence in the immunoreceptors interacts with the membrane surface and modulates the signaling function of its host receptor.

Cell adhesion receptors

Like immunoreceptors, cell adhesion receptors typically contain no intrinsic kinase domains nor associate constitutively with intracellular kinases. Comparing to immunoreceptors, cell adhesion receptors vary greatly from one another in their signaling mechanisms due to the diverse sequence motifs in the cytoplasmic domains. Correspondingly, the JM sequence may participate in the transmembrane signaling of adhesion receptors via diverse mechanisms.

Platelet-specific integrin α IIb β 3 embodies the best-characterized inside-out activation mechanism of a cell adhesion receptor⁵⁰. Early studies identified mutations in the JM regions of both α IIb and β 3 subunits that can activate integrin in the absence of an intracellular signal, suggesting that there is an electrostatic interaction between the JM

regions to keep the integrin in the inactive state⁵¹. Recent studies have revealed that the TM-JM region of $\beta 3$ forms a continuous TM helix whereas the αIIb JM region may not be in a helical conformation⁵²⁻⁵⁴. The interaction between the JM regions is relatively weak and may be modulated by the TM helical association, the tilt angle of the adjoining TM helix, the membrane environment, and the association of intracellular proteins to the $\beta 3$ cytoplasmic domain⁵⁵⁻⁵⁸. In addition, the $\beta 3$ cytoplasmic domain has been suggested to bind the membrane surface. Upon membrane association, the $\beta 3$ cytoplasmic domain, which contains two NPxY sequence motifs, becomes more ordered and helical, modulating possibly the phosphorylation state of tyrosine residues therein⁵⁹.

Upon ligand binding many adhesion receptors transmit outside-in signals across the plasma membrane. A frequently cited mechanism is the clustering of adhesion receptors. In many cases, the transmembrane domain mediates the self-association of the host receptor or the association with other co-receptors⁶⁰⁻⁶⁷. The intracellular JM region can also participate or modulate such association^{68, 69}. In some cases, the JM region, instead of the transmembrane helix, appears to drive the association^{70, 71}. In addition, the intracellular JM region in many adhesion receptors is enriched with basic residues and involved in the efficient surface expression of the receptor or the association with intracellular signaling or cytoskeletal proteins^{72, 73}. Nevertheless, how the JM region propagates or modulates the outside-in signaling of adhesion receptors remains to be delineated.

Summary

Recent reports have provided clear evidence supporting the involvement of the JM region in the signaling of many diverse transmembrane receptors. It also appears that the JM regions across different types of receptors often share similar mechanisms in mediating the signaling pathway. For instance, the association of JM region with the anionic membrane surface, and its participation or facilitation of self-association in conjunction with the adjoining TM helix, has been reported for various transmembrane receptors. The new appreciation of the functional importance of JM region also brings up a set of questions related to its structural and energetic aspects⁷⁴. For instance, the Gibbs free energy of TM helical association in the membrane is measured in the unit of protein/lipid mole fraction rather than molar concentration (*i.e.* protein/water mole fraction)⁷⁵. It would be challenging to describe and measure the energetics of association of a TM-JM fragment in which the JM region self-associates in the aqueous phase and cooperates with the TM domain to mediate oligomerization of the fragment. It may become more complicated if one is to include the free energy required to dissociate the aforementioned JM region from the membrane surface prior to its self-association. Future investigation will produce more exciting and revealing insights on the mechanisms of JM dynamics during transmembrane receptor signaling.

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References

1. Hulko M, Berndt F, Gruber M, Linder JU, Truffault V, Schultz A, Martin J, Schultz JE, Lupas AN, Coles M. The HAMP domain structure implies helix rotation in transmembrane signaling. *Cell*. 2006; 126:929–940. [PubMed: 16959572]
2. Matthews EE, Zoonens M, Engelman DM. Dynamic helix interactions in transmembrane signaling. *Cell*. 2006; 127:447–450. [PubMed: 17081964]
3. Gifford JL, Ishida H, Vogel HJ. Structural Insights into Calmodulin-regulated L-selectin Ectodomain Shedding. *J. Biol. Chem*. 2012; 287:26513–26527. [PubMed: 22711531]
4. Deng W, Putkey JA, Li R. Calmodulin adopts an extended conformation when interacting with L-selectin in membranes. *PLoS One*. 2013; 8:e62861. [PubMed: 23658780]
5. Ottemann KM, Xiao W, Shin Y-K, Koshland DE Jr. A piston model for transmembrane signaling of the aspartate receptor. *Science*. 1999; 285:1751–1754. [PubMed: 10481014]
6. Murphy OJ 3rd, Kovacs FA, Sicard EL, Thompson LK. Site-directed solid-state NMR measurement of a ligand-induced conformational change in the serine bacterial chemoreceptor. *Biochemistry*. 2001; 40:1358–1366. [PubMed: 11170463]
7. Yarden Y, Schlessinger J. Epidermal growth factor induces rapid, reversible aggregation of the purified epidermal growth factor receptor. *Biochemistry*. 1987; 26:1443–1451. [PubMed: 3494473]
8. Yarden Y, Schlessinger J. Self-phosphorylation of epidermal growth factor receptor: evidence for a model of intermolecular allosteric activation. *Biochemistry*. 1987; 26:1434–1442. [PubMed: 3494472]
9. Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell*. 2000; 103:211–225. [PubMed: 11057895]
10. Endres NF, Barros T, Cantor AJ, Kuriyan J. Emerging concepts in the regulation of the EGF receptor and other receptor tyrosine kinases. *Trends Biochem Sci*. 2014; 39:437–446. [PubMed: 25242369]
11. Hubbard SR. Juxtamembrane autoinhibition in receptor tyrosine kinases. *Nat Rev Mol Cell Biol*. 2004; 5:464–471. [PubMed: 15173825]
12. 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; 12:541–552. [PubMed: 14527402]
13. Bargmann CI, Hung MC, Weinberg RA. Multiple independent activations of the neu oncogene by a point mutation altering the transmembrane domain of p185. *Cell*. 1986; 45:649–657. [PubMed: 2871941]
14. Cao H, Bangalore L, Bormann BJ, Stern DF. A subdomain in the transmembrane domain is necessary for p185neu* activation. *EMBO J*. 1992; 11:923–932. [PubMed: 1347745]
15. Smith SO, Smith CS, Bormann BJ. Strong hydrogen bonding interactions involving a buried glutamic acid in the transmembrane sequence of the neu/erbB-2 receptor. *Nat. Struct. Biol*. 1996; 3:252–258. [PubMed: 8605627]
16. Matsushita C, Tamagaki H, Miyazawa Y, Aimoto S, Smith SO, Sato T. Transmembrane helix orientation influences membrane binding of the intracellular juxtamembrane domain in Neu receptor peptides. *Proc. Natl. Acad. Sci. USA*. 2013; 110:1646–1651. [PubMed: 23319611]
17. McLaughlin S, Smith SO, Hayman MJ, Murray D. An electrostatic engine model for autoinhibition and activation of the epidermal growth factor receptor (EGFR/ErbB) family. *The Journal of general physiology*. 2005; 126:41–53. [PubMed: 15955874]
18. 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; 137:1293–1307. [PubMed: 19563760]
19. Endres NF, Das R, Smith AW, Arkhipov A, Kovacs E, Huang Y, Pelton JG, Shan Y, Shaw DE, Wemmer DE, Groves JT, Kuriyan J. Conformational coupling across the plasma membrane in activation of the EGF receptor. *Cell*. 2013; 152:543–556. [PubMed: 23374349]
20. Arkhipov A, Shan Y, Das R, Endres NF, Eastwood MP, Wemmer DE, Kuriyan J, Shaw DE. Architecture and membrane interactions of the EGF receptor. *Cell*. 2013; 152:557–569. [PubMed: 23374350]

21. Tamagaki H, Furukawa Y, Yamaguchi R, Hojo H, Aimoto S, Smith SO, Sato T. Coupling of transmembrane helix orientation to membrane release of the juxtamembrane region in FGFR3. *Biochemistry*. 2014; 53:5000–5007. [PubMed: 25010350]
22. Yu X, Sharma KD, Takahashi T, Iwamoto R, Mekada E. Ligand-independent dimer formation of epidermal growth factor receptor (EGFR) is a step separable from ligand-induced EGFR signaling. *Mol. Biol. Cell*. 2002; 13:2547–2557. [PubMed: 12134089]
23. Nagy P, Claus J, Jovin TM, Arndt-Jovin DJ. Distribution of resting and ligand-bound ErbB1 and ErbB2 receptor tyrosine kinases in living cells using number and brightness analysis. *Proc. Natl. Acad. Sci. USA*. 2010; 107:16524–16529. [PubMed: 20813958]
24. Noordeen NA, Carafoli F, Hohenester E, Horton MA, Leitinger B. A transmembrane leucine zipper is required for activation of the dimeric receptor tyrosine kinase DDR1. *J. Biol. Chem*. 2006; 281:22744–22751. [PubMed: 16774916]
25. Kim D, Ko P, You E, Rhee S. The intracellular juxtamembrane domain of discoidin domain receptor 2 (DDR2) is essential for receptor activation and DDR2-mediated cancer progression. *Int J Cancer*. 2014; 135:2547–2557. [PubMed: 24740739]
26. Constantinescu SN, Keren T, Socolovsky M, Nam H, Henis YI, Lodish HF. Ligand-independent oligomerization of cell-surface erythropoietin receptor is mediated by the transmembrane domain. *Proc. Natl. Acad. Sci. USA*. 2001; 98:4379–4384. [PubMed: 11296286]
27. Ross RJ, Leung KC, Maamra M, Bennett W, Doyle N, Waters MJ, Ho KK. Binding and functional studies with the growth hormone receptor antagonist, B2036-PEG (pegvisomant), reveal effects of pegylation and evidence that it binds to a receptor dimer. *The Journal of clinical endocrinology and metabolism*. 2001; 86:1716–1723. [PubMed: 11297608]
28. Schuster B, Meinert W, Rose-John S, Kallen KJ. The human interleukin-6 (IL-6) receptor exists as a preformed dimer in the plasma membrane. *FEBS Lett*. 2003; 538:113–116. [PubMed: 12633863]
29. Sykes AM, Palstra N, Abankwa D, Hill JM, Skeldal S, Matusica D, Venkatraman P, Hancock JF, Coulson EJ. The effects of transmembrane sequence and dimerization on cleavage of the p75 neurotrophin receptor by gamma-secretase. *J. Biol. Chem*. 2012; 287:43810–43824. [PubMed: 23105112]
30. Brooks AJ, Dai W, O'Mara ML, Abankwa D, Chhabra Y, Pelekanos RA, Gardon O, Tunny KA, Blucher KM, Morton CJ, Parker MW, Sierecki E, Gambin Y, Gomez GA, Alexandrov K, Wilson IA, Doxastakis M, Mark AE, Waters MJ. Mechanism of activation of protein kinase JAK2 by the growth hormone receptor. *Science*. 2014; 344:1249783. [PubMed: 24833397]
31. Erickson-Miller CL, DeLorme E, Tian SS, Hopson CB, Stark K, Giampa L, Valoret EI, Duffy KJ, Luengo JL, Rosen J, Miller SG, Dillon SB, Lamb P. Discovery and characterization of a selective, nonpeptidyl thrombopoietin receptor agonist. *Exp. Hematol*. 2005; 33:85–93. [PubMed: 15661401]
32. Kim MJ, Park SH, Opella SJ, Marsilje TH, Michellys PY, Seidel HM, Tian SS. NMR structural studies of interactions of a small, nonpeptidyl tpo mimic with the thrombopoietin receptor extracellular juxtamembrane and transmembrane domains. *J. Biol. Chem*. 2007; 282:14253–14261. [PubMed: 17369254]
33. Matthews EE, Thevenin D, Rogers JM, Gotow L, Lira PD, Reiter LA, Brissette WH, Engelman DM. Thrombopoietin receptor activation: transmembrane helix dimerization, rotation, and allosteric modulation. *FASEB J*. 2011; 25:2234–2244. [PubMed: 21402716]
34. Lu X, Gross AW, Lodish HF. Active conformation of the erythropoietin receptor: random and cysteine-scanning mutagenesis of the extracellular juxtamembrane and transmembrane domains. *J. Biol. Chem*. 2006; 281:7002–7011. [PubMed: 16414957]
35. Staerk J, Lacout C, Sato T, Smith SO, Vainchenker W, Constantinescu SN. An amphipathic motif at the transmembrane-cytoplasmic junction prevents autonomous activation of the thrombopoietin receptor. *Blood*. 2006; 107:1864–1871. [PubMed: 16249382]
36. Defour JP, Itaya M, Gryshkova V, Brett IC, Pecquet C, Sato T, Smith SO, Constantinescu SN. Tryptophan at the transmembrane-cytosolic junction modulates thrombopoietin receptor dimerization and activation. *Proc. Natl. Acad. Sci. USA*. 2013; 110:2540–2545. [PubMed: 23359689]

37. Constantinescu SN, Huang LJ, Nam H, Lodish HF. The erythropoietin receptor cytosolic juxtamembrane domain contains an essential, precisely oriented, hydrophobic motif. *Mol. Cell.* 2001; 7:377–385. [PubMed: 11239466]
38. Sheets ED, Holowka D, Baird B. Membrane organization in immunoglobulin E receptor signaling. *Curr. Opin. Chem. Biol.* 1999; 3:95–99. [PubMed: 10021405]
39. Janes PW, Ley SC, Magee AI, Kabouridis PS. The role of lipid rafts in T cell antigen receptor (TCR) signalling. *Semin Immunol.* 2000; 12:23–34. [PubMed: 10723795]
40. Locke D, Chen H, Liu Y, Liu C, Kahn ML. Lipid rafts orchestrate signaling by the platelet receptor glycoprotein VI. *J. Biol. Chem.* 2002; 277:18801–18809. [PubMed: 11844795]
41. Xu C, Gagnon E, Call ME, Schnell JR, Schwieters CD, Carman CV, Chou JJ, Wucherpfennig KW. Regulation of T cell receptor activation by dynamic membrane binding of the CD3epsilon cytoplasmic tyrosine-based motif. *Cell.* 2008; 135:702–713. [PubMed: 19013279]
42. Aivazian D, Stern LJ. Phosphorylation of T cell receptor zeta is regulated by a lipid dependent folding transition. *Nat. Struct. Biol.* 2000; 7:1023–1026. [PubMed: 11062556]
43. Zhang H, Cordoba SP, Dushek O, van der Merwe PA. Basic residues in the T-cell receptor zeta cytoplasmic domain mediate membrane association and modulate signaling. *Proc. Natl. Acad. Sci. USA.* 2011; 108:19323–19328. [PubMed: 22084078]
44. DeFord-Watts LM, Dougall DS, Belkaya S, Johnson BA, Eitson JL, Roybal KT, Barylko B, Albanesi JP, Wulfig C, van Oers NS. The CD3 zeta subunit contains a phosphoinositide-binding motif that is required for the stable accumulation of TCR-CD3 complex at the immunological synapse. *J. Immunol.* 2011; 186:6839–6847. [PubMed: 21543646]
45. Shi X, Bi Y, Yang W, Guo X, Jiang Y, Wan C, Li L, Bai Y, Guo J, Wang Y, Chen X, Wu B, Sun H, Liu W, Wang J, Xu C. Ca²⁺ regulates T-cell receptor activation by modulating the charge property of lipids. *Nature.* 2013; 493:111–115. [PubMed: 23201688]
46. Sigalov AB, Aivazian DA, Uversky VN, Stern LJ. Lipid-binding activity of intrinsically unstructured cytoplasmic domains of multichain immune recognition receptor signaling subunits. *Biochemistry.* 2006; 45:15731–15739. [PubMed: 17176095]
47. Kim J, Shishido T, Jiang X, Aderem A, McLaughlin S. Phosphorylation, high ionic strength, and calmodulin reverse the binding of MARCKS to phospholipid vesicles. *J. Biol. Chem.* 1994; 269:28214–28219. [PubMed: 7961759]
48. Heo WD, Inoue T, Park WS, Kim ML, Park BO, Wandless TJ, Meyer T. PI(3,4,5)P₃ and PI(4,5)P₂ lipids target proteins with polybasic clusters to the plasma membrane. *Science.* 2006; 314:1458–1461. [PubMed: 17095657]
49. Paddock C, Lytle BL, Peterson FC, Holyst T, Newman PJ, Volkman BF, Newman DK. Residues within a lipid-associated segment of the PECAM-1 cytoplasmic domain are susceptible to inducible, sequential phosphorylation. *Blood.* 2011; 117:6012–6023. [PubMed: 21464369]
50. Kim C, Ye F, Ginsberg MH. Regulation of integrin activation. *Annu Rev Cell Dev Biol.* 2011; 27:321–345. [PubMed: 21663444]
51. Hughes PE, Diaz-Gonzalez F, Leong L, Wu C, McDonald JA, Shattil SJ, Ginsberg MH. Breaking the integrin hinge. A defined structural constraint regulates integrin signaling. *J. Biol. Chem.* 1996; 271:6571–6574. [PubMed: 8636068]
52. Li R, Babu CR, Valentine K, Lear JD, Wand AJ, Bennett JS, DeGrado WF. Characterization of the monomeric form of the transmembrane and cytoplasmic domains of integrin b3 subunit by NMR spectroscopy. *Biochemistry.* 2002; 41:15618–15624. [PubMed: 12501190]
53. Lau TL, Kim C, Ginsberg MH, Ulmer TS. The structure of the integrin alphaIIb beta3 transmembrane complex explains integrin transmembrane signalling. *EMBO J.* 2009; 28:1351–1361. [PubMed: 19279667]
54. Yang J, Ma YQ, Page RC, Misra S, Plow EF, Qin J. Structure of an integrin alphaIIb beta3 transmembrane-cytoplasmic heterocomplex provides insight into integrin activation. *Proc. Natl. Acad. Sci. USA.* 2009; 106:17729–17734. [PubMed: 19805198]
55. Vinogradova O, Vaynberg J, Kong X, Haas TA, Plow EF, Qin J. Membrane-mediated structural transitions at the cytoplasmic face during integrin activation. *Proc. Natl. Acad. Sci. USA.* 2004; 101:4094–4099. [PubMed: 15024114]

56. Li W, Metcalf DG, Gorelik R, Li R, Mitra N, Nanda V, Law PB, Lear JD, DeGrado WF, Bennett JS. A push-pull mechanism for regulating integrin function. *Proc. Natl. Acad. Sci. USA.* 2005; 102:1424–1429. [PubMed: 15671157]
57. Anthis NJ, Wegener KL, Ye F, Kim C, Goult BT, Lowe ED, Vakonakis I, Bate N, Critchley DR, Ginsberg MH, Campbell ID. The structure of an integrin/talin complex reveals the basis of inside-out signal transduction. *EMBO J.* 2009; 28:3623–3632. [PubMed: 19798053]
58. Kim C, Schmidt T, Cho EG, Ye F, Ulmer TS, Ginsberg MH. Basic amino-acid side chains regulate transmembrane integrin signalling. *Nature.* 2012; 481:209–213.
59. Metcalf DG, Moore DT, Wu Y, Kielec JM, Molnar K, Valentine KG, Wand AJ, Bennett JS, DeGrado WF. NMR analysis of the α IIb β 3 cytoplasmic interaction suggests a mechanism for integrin regulation. *Proc. Natl. Acad. Sci. USA.* 2010; 107:22481–22486. [PubMed: 21156831]
60. Ushiyama S, Laue TM, Moore KL, Erickson HP, McEver RP. Structural and functional characterization of monomeric soluble P-selectin and comparison with membrane P-selectin. *J. Biol. Chem.* 1993; 268:15229–15237. [PubMed: 7686912]
61. Epperson TK, Patel KD, McEver RP, Cummings RD. Noncovalent association of P-selectin glycoprotein ligand-1 and minimal determinants for binding to P-selectin. *J. Biol. Chem.* 2000; 275:7839–7853. [PubMed: 10713099]
62. Li R, Gorelik R, Nanda V, Law PB, Lear JD, DeGrado WF, Bennett JS. Dimerization of the transmembrane domain of integrin α IIb subunit in cell membranes. *J. Biol. Chem.* 2004; 279:26666–26673. [PubMed: 15067009]
63. Choi S, Lee E, Kwon S, Park H, Yi JY, Kim S, Han IO, Yun Y, Oh ES. Transmembrane domain-induced oligomerization is crucial for the functions of syndecan-2 and syndecan-4. *J. Biol. Chem.* 2005; 280:42573–42579. [PubMed: 16253987]
64. Dews IC, Mackenzie KR. Transmembrane domains of the syndecan family of growth factor coreceptors display a hierarchy of homotypic and heterotypic interactions. *Proc. Natl. Acad. Sci. USA.* 2007; 104:20782–20787. [PubMed: 18093920]
65. Luo SZ, Li R. Specific heteromeric association of four transmembrane peptides derived from platelet glycoprotein Ib-IX complex. *J. Mol. Biol.* 2008; 382:448–457. [PubMed: 18674540]
66. Berger BW, Kulp DW, Span LM, DeGrado JL, Billings PC, Senes A, Bennett JS, DeGrado WF. Consensus motif for integrin transmembrane helix association. *Proc. Natl. Acad. Sci. USA.* 2010; 107:703–708. [PubMed: 20080739]
67. Godfroy JI 3rd, Roostan M, Moroz YS, Korendovych IV, Yin H. Isolated Toll-like receptor transmembrane domains are capable of oligomerization. *PLoS One.* 2012; 7:e48875. [PubMed: 23155421]
68. Miner JJ, Shao B, Wang Y, Chichili GR, Liu Z, Klopocki AG, Yago T, McDaniel JM, Rodgers W, Xia L, McEver RP. Cytoplasmic domain of P-selectin glycoprotein ligand-1 facilitates dimerization and export from the endoplasmic reticulum. *J. Biol. Chem.* 2011; 286:9577–9586. [PubMed: 21220419]
69. Barton R, Palacio D, Iovine MK, Berger BW. A cytosolic juxtamembrane interface modulates plexin α 3 oligomerization and signal transduction. *PLoS One.* 2015; 10:e0116368. [PubMed: 25565389]
70. Su PC, Berger BW. Identifying Key Juxtamembrane Interactions in Cell Membranes Using AraC-based Transcriptional Reporter Assay (AraTM). *J. Biol. Chem.* 2012; 287:31515–31526. [PubMed: 22822084]
71. Deng W, Cho S, Su PC, Berger BW, Li R. Membrane-enabled dimerization of the intrinsically disordered cytoplasmic domain of ADAM10. *Proc. Natl. Acad. Sci. USA.* 2014; 111:15987–15992. [PubMed: 25349418]
72. Wang Y, Yago T, Zhang N, Abdisalaam S, Alexandrakis G, Rodgers W, McEver RP. Cytoskeletal Regulation of CD44 Membrane Organization and Interactions with E-selectin. *J. Biol. Chem.* 2014; 289:35159–35171. [PubMed: 25359776]
73. Deng W, Cho S, Li R. FERM Domain of Moesin Desorbs the Basic-Rich Cytoplasmic Domain of I-Selectin from the Anionic Membrane Surface. *J. Mol. Biol.* 2013; 425:3549–3562. [PubMed: 23796515]

74. Grigoryan G, Moore DT, DeGrado WF. Transmembrane communication: general principles and lessons from the structure and function of the M2 proton channel, K(+) channels, and integrin receptors. *Annu Rev Biochem.* 2011; 80:211–237. [PubMed: 21548783]
75. MacKenzie KR, Fleming KG. Association energetics of membrane spanning alpha-helices. *Curr. Opin. Struct. Biol.* 2008; 18:412–419. [PubMed: 18539023]

Figure 1.

Examples of juxtamembrane (JM) modulation of transmembrane signaling. Only the transmembrane (TM) domain and its adjoining JM region are shown in the illustrations. (A) In the inactive EGFR, the JM region is associated with the anionic membrane surface via its basic-rich sequence. Ligand binding induces the dimerization of the TM helix and the formation of an antiparallel JM dimer, which facilitates formation of the asymmetric dimer of the kinase domains. (B) Inactive GHR is a constitutive dimer, partly mediated by its TM helices. Ligand binding in the extracellular domain induces a rotation of the TM helix and rearrangement of the TM dimer, resulting in the separation of TM sequence in the intracellular side. The intracellular JM sequence may also interact with the membrane and modulates the activation of the cognate Janus kinases (JAK). (C) In the inactive CD3ε, the basic-rich JM sequence binds to the plasma membrane, facilitating the burial of the nearby tyrosine residue in the ITAM sequence motif. Ligand binding, increase of intracellular calcium level, or mutations in the JM sequence induces the dissociation of JM sequence from the anionic membrane surface and facilitates the phosphorylation of tyrosine in the cytoplasmic domain.