

Activation of transmembrane cell-surface receptors via a common mechanism? The “rotation model”

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It has long been thought that transmembrane cell-surface receptors, such as receptor tyrosine kinases and cytokine receptors, among others, are activated by ligand binding through ligand-induced dimerization of the receptors. However, there is growing evidence that prior to ligand binding, various transmembrane receptors have a preformed, yet inactive, dimeric structure on the cell surface. Various studies also demonstrate that during transmembrane signaling, ligand binding to the extracellular domain of receptor dimers induces a rotation of transmembrane domains, followed by rearrangement and/or activation of intracellular domains. The paper here describes transmembrane cell-surface receptors that are known or proposed to exist in dimeric form prior to ligand binding, and discusses how these preformed dimers are activated by ligand binding.

Keywords:

■ cytokine; dimerization; ligand binding; preformed dimer; transmembrane signaling; tyrosine kinase

Introduction

Transmembrane, cell-surface receptors transmit extracellular signals across

cell membranes to the cytoplasm, and include receptor tyrosine kinases (RTKs) and cytokine receptors among many others. Cell-surface receptors

typically consist of an extracellular domain (ECD) and an intracellular domain (ICD) separated by a single transmembrane domain (TMD), with the exception of bacterial receptors such as the aspartate receptor (Tar) and the serine receptor (Tsr), which have another TMD at their amino termini. Ligand binding to their ECDs often regulates kinases that are either integrated into the receptor ICD, or physically associated with the ICD. Apart from receptors that initiate signaling pathways inside cells via tyrosine phosphorylation, there are receptors in bacteria, fungi, and plants that phosphorylate histidine residues upon ligand binding. Furthermore, natriuretic peptide receptors, which are receptor-type guanylyl cyclases, produce cGMP upon peptide binding. There are also receptors that recruit adaptor/effector proteins through protein-protein interactions upon ligand binding.

There are two major, mutually exclusive concepts to explain activation of transmembrane, cell-surface receptors. Ligand binding induces either (i) dimerization of receptors, or (ii) rearrangement of constitutively preformed dimeric receptors. The former mechanism, known as ligand-induced receptor dimerization, was first proposed for the epidermal growth factor receptor (EGFR; also called ErbB1 or HER1) almost three decades ago [1–3]. In this “dimerization model,” the receptor is thought to exist in monomeric form on the cell surface prior to ligand binding, which induces receptor

DOI 10.1002/bies.201500041

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Abbreviations:

BRET, bioluminescence resonance energy transfer; **ECD**, extracellular domain; **EGFR**, epidermal growth factor receptor; **Eph**, ephrin receptor; **EpoR**, erythropoietin receptor; **ErbB**, originally named because of the homology to the erythroblastoma viral gene product, v-erbB; **FGFR**, fibroblast growth factor receptor; **FnIII**, fibronectin type III; **FRET**, Förster resonance energy transfer;

GCY-14, receptor-type guanylyl cyclase-14; **GHR**, growth hormone receptor; **ICD**, intracellular domain; **IGF1R**, insulin-like growth factor-1 receptor; **IL-6R**, interleukin 6 receptor; **IL-12R**, interleukin 12 receptor; **IR**, insulin receptor; **IRR**, insulin receptor-related receptor; **JAK**, Janus kinase; **LepR**, leptin receptor; **NPRA**, natriuretic peptide receptor A; **p75^{NTR}**, neurotrophin receptor; **PRLR**, prolactin receptor; **RTK**, receptor tyrosine kinase; **STAT**, signal transducers and activators of transcription; **Tar**, bacterial aspartate receptor; **TLR**, toll-like receptor; **TMD**, transmembrane domain; **TNFR**, tumor necrosis factor receptor; **TpoR**, thrombopoietin receptor; **Trk**, tropomyosin-related kinase receptor; **Tsr**, bacterial serine receptor.

dimerization. According to this model, ICDs of dimerized receptors are brought into close proximity to allow receptor trans-autophosphorylation, subsequent tyrosine kinase activation, and initiation of downstream signaling pathways [4, 5]. This mechanism has been proposed for activation of many other cell-surface receptors, including receptor tyrosine kinases and cytokine receptors. In contrast, the insulin receptor (IR), insulin-like growth factor-1 receptor (IGF1R), and insulin receptor-related receptor (IRR) have covalent, preformed, dimeric structures linked by cysteine disulfide bridges [6, 7]. Likewise, there is growing evidence that prior to ligand binding, various transmembrane receptors exist in preformed, yet inactive, dimeric form on the cell surface (Table 1).

A variety of receptors exist in dimeric form prior to ligand binding

Receptor tyrosine kinases

The human RTK superfamily consists of 58 proteins grouped into 20 subfamilies [8]. RTKs are integral membrane proteins with a single TMD, and their N-terminal ECDs are generally composed of various structural modules with multiple, intrachain, disulfide bonds, and numerous N-linked glycosylation sites. Their ICDs have tyrosine kinase domains flanked by intracellular, juxtamembrane regions, and C-terminal tails, which differ in size and tyrosine content among family members. Ligand binding to the ECDs results in elevation of their tyrosine

kinase activity and in selective trans-autophosphorylation of tyrosine residues. Some of these sites are involved in maintaining active conformations of the kinases, while others become docking sites for various adaptor/effector scaffold proteins and enzymes. All RTKs, except for the IR family, are expressed as single protomers. IR family members, comprising IR, IGF1R, and IRR, are also expressed as single subunits, but they undergo processing into two α and two β polypeptide chains that are assembled into a heterotetramer, or an $(\alpha\beta)_2$ homodimer, stabilized by disulfide bonds.

EGFR (ErbB) family

The ErbB receptor family consists of EGFR, ErbB2 (also known as Neu/HER2), ErbB3 (HER3), and ErbB4 (HER4), and the receptors play crucial roles in cell growth, differentiation, survival, and migration. A number of studies demonstrate that prior to ligand binding, ErbB receptors exist in dimeric form on the cell surface (see [9], and references therein). Chemical cross-linking showed that >80% of EGFR molecules were dimeric in the absence of bound ligand [10]. Förster resonance energy transfer (FRET) [11–14] and fluorescence correlation spectroscopic analyses [13, 15, 16] further demonstrated that preformed EGFR and ErbB2 dimers are present at physiological expression levels on surfaces of living cells. Single-molecule observations using total internal reflection fluorescence microscopy with oblique illumination also supports the existence of receptor dimers [17]. Fluorescent protein fragment complementation indicates that all the members of the ErbB family exist in dimeric form [18]. This is consistent with results of reversible firefly luciferase fragment complementation analysis, showing that 100% of EGFR and ErbB3 receptor molecules exist as dimers [19–21], since luciferase activity did not increase after addition of EGF to the cell culture. Depending on methods used, dimer-to-monomer ratios vary from 40 to 100%. Considering the inefficiency of chemical cross-linking [22] and of fluorescent protein folding [23–25], these ratios are likely to be underestimated. However, when EGFR mutants with

Table 1. Transmembrane cell-surface receptors that exist or are proposed to exist in dimeric form prior to ligand binding

	References	Rotation angle and reference
Receptor tyrosine kinases		
EGFR	[10–21]	[~140°: 10]
ErbB2	[13, 18, 80]	
ErbB3	[18, 21]	
ErbB4	[18]	
EphA1	[40, 41]	
EphA2	[38, 39, 41]	[60°: 41]
EphA3	[37]	
FGFR3	[43]	
IGF1R	[32]	
IR	[32]	
IRR	[33]	
MET	[42]	
TrkA	[28]	
TrkB	[29]	
VEGFR2		[180°: 99]
Cytokine receptors		
EpoR	[50, 51]	[~100°: 91, 92]
GHR	[48, 49]	[~40°: 93, 94; ~45°: 95]
IL-6R	[60]	
IL-12R	[58]	
LepR	[55–57]	
p75 ^{NTR}	[30]	
PRLR	[52]	
TNFR	[62]	
TpoR		[~100°: 53]
Other cell-surface receptors		
EnvZ	[69]	
GCY-14	[65]	
LINGO-1	[66]	
NPRA	[64]	[40°: 96]
Tar	[67]	[~50°: 74]
TLR9	[63]	
Tsr	[68]	

Receptors in which TMDs have been proposed to rotate during signaling are indicated with bold letters, with or without rotation angles and reference(s).

cysteine substitutions at different locations in the TMD were expressed in a murine pre-B lymphocyte line, Ba/F3, disulfide cross-linking of the receptors was observed only in the presence of EGF [26]. This result is inconsistent with the previous result in which similar EGFR or ErbB2 constructs with a cysteine substitution spontaneously formed dimers in the absence of bound EGF, when expressed in mouse fibroblast B82 cells or monkey fibroblast-like COS-7 cells, respectively [10, 27]. All disulfide cross-linkings of EGFR extracellular juxtamembrane regions induced autophosphorylation to various extents, depending upon where cysteines had been replaced. These phosphorylated receptors were internalized and degraded in the absence of bound EGF, and their efficiency is likely to be cell type-dependent. Therefore, it would be necessary to observe the spontaneous dimerization of the cysteine-replaced EGFR mutants expressed in Ba/F3 by inhibiting their endocytosis.

Neurotrophin receptors

There is also evidence that many non-ErbB family RTKs exist as dimers in the absence of bound ligand. Chemical cross-linking and firefly luciferase complementation analyses demonstrate that the neurotrophin receptors, TrkA and TrkB, which bind nerve growth factor and brain-derived neurotrophic factor, respectively, exist in dimeric form [28, 29]. Luciferase activity did not increase with addition of ligand to the cell cultures, indicating that 100% of these receptors have preformed dimeric structures. p75^{NTR} is a member of the tumor necrosis factor (TNF) receptor (TNFR) superfamily. It does not have kinase activity and binds all neurotrophins with low affinity. It exists as a disulfide-linked dimer owing to a highly conserved cysteine in its TMD [30].

IR and Eph families, and others

IR and IGF1R, which play critical roles in metabolism and cell growth, and IRR, which is an extracellular alkaline pH sensor [31], are covalent, disulfide-linked ($\alpha\beta$)₂ dimers comprising two

extracellular α -subunits that contain ligand-binding domains and two transmembrane β -subunits that possess intracellular kinase domains [32, 33]. There is also evidence for the existence of a disulfide-linked ($\alpha\beta$)₂ hybrid dimeric receptor (IR::IGF1R), which is composed of an IR $\alpha\beta$ hemireceptor and an IGF1R $\alpha\beta$ hemireceptor [34, 35]. Eph RTKs mediate contact-dependent, cell-cell communication by interacting with surface-associated ligands (ephrin) on neighboring cells [36]. EphA3, which is essential for cell guidance during embryogenesis, clusters as a result of EphA3-EphA3 interactions, which are independent of ligand binding [37]. Similarly, EphA2 also constitutively forms dimers without bound ligand [38, 39]. Consistently, EphA1 and EphA2 TMDs spontaneously form homodimers in lipid [40, 41]. The MET receptor for hepatocyte growth factor/scatter factor, which is essential during embryonic development and plays an important role during cancer metastasis and tissue regeneration, has been shown to exist as a dimer, based on photobleaching experiments using single-molecule fluorescence microscopy [42]. TMDs of fibroblast growth factor receptor 3 (FGFR3), which is a negative regulator of bone growth, and which is critically important for skeletal development, interact as dimers and this interaction persists if their ECDs are present [43].

Cytokine receptors

There are more than 30 Class I cytokine receptors [44] and at least 12 Class II cytokine receptors [45] that activate JAK1 (Janus kinase 1), JAK2, JAK3, or TYK2 (tyrosine kinase 2). Class I and Class II receptors are distinguished by the position of class-specific cysteine residues, and by the presence of a highly conserved “WSXWS” motif in the carboxyl terminal half of Class I receptor ECDs. Cytokine receptors comprise two receptor subunits, each of which associates with a JAK monomer. Upon cytokine binding, the receptor activates the associated JAKs, which in turn phosphorylate tyrosine residues within the receptor ICD. The phosphorylated tyrosine residues serve as docking sites for downstream adaptor and effector proteins, which include the STAT

(signal transducers and activators of transcription) proteins. A series of landmark publications gave rise to the textbook view that ligand binding initiates cytokine receptor dimerization, which then leads to activation of a tyrosine kinase associated with the receptor [46, 47]. However, at least nine distinct cytokine receptors have been shown or proposed to exist in preformed dimeric form (Table 1).

The growth hormone receptor (GHR) is required for postnatal growth, as well as for lipid and carbohydrate metabolism. It dimerizes in the endoplasmic reticulum before reaching the cell surface [48, 49]. Ligand-independent oligomerization of the cell-surface erythropoietin receptor (EpoR), which is crucial for production of mature red blood cells, has also been observed by immunofluorescence co-patching [50]. Consistently, the crystal structure of the EpoR ECD is homodimeric in the absence of bound ligand [51]. The prolactin receptor (PRLR) mediates effects of prolactin, which stimulates growth and differentiation of mammary epithelium and initiation and maintenance of lactation. Co-immunoprecipitation assays were used to confirm its ligand-independent dimerization. In this dimerization process, the TMDs play a significant role [52]. The thrombopoietin receptor (TpoR) regulates the proliferation of multipotent, hematopoietic bone marrow stem cells, their differentiation into mature megakaryocytes, and production of platelets in response to thrombopoietin binding. Using a combination of cysteine cross-linking, alanine-scanning mutagenesis, and computational simulations, it was shown that TpoR TMDs dimerize strongly in membranes in the absence of bound ligand [53].

The leptin receptor (LepR) plays a central role in control of body weight and energy homeostasis. LepR shows great similarity to the interleukin 6 (IL-6) signaling receptor chain glycoprotein 130 (gp130), the granulocyte colony-stimulating factor receptor, and the leukemia inhibitory factor receptor, and uses JAK2 and STAT3 for its signaling pathway [54]. In cell membranes, LepR assembles as preformed dimers or oligomers, as evidenced by a high basal signal in the absence of leptin in analysis of differently tagged LepRs by

co-immunoprecipitation, bioluminescence resonance energy transfer (BRET), and FRET [55–57]. IL-12 is a heterodimeric cytokine composed of two disulfide-bonded, glycoprotein subunits, and has pleiotropic effects on NK and T cells, which are mediated through IL-12 receptors (IL-12Rs). When IL-12Rs were expressed in COS cells, they expressed both monomers and disulfide-linked dimers or oligomers on their surfaces in the absence of IL-12, among which only the IL-12R dimers/oligomers, but not the monomers, bind IL-12 [58]. Upon IL-6 binding, the IL-6 receptor (IL-6R), which is essential for regenerative and anti-bacterial effects of IL-6 [59], activates the gp130 homodimer, leading to initiation of JAK/STAT signaling. Co-immunoprecipitation experiments with two differently tagged IL-6R variants expressed in COS-7 cells showed that an IL-6R dimer exists in the plasma membrane in the absence of IL-6 [60].

TNF is a cytokine for immune responses and inflammation, and is a homotrimer with a molecular mass of 52 kDa. TNF binds with high affinity to two type-I transmembrane receptors: TNFR1, which is activated by both soluble TNF and transmembrane TNF, and TNFR2, which is activated mainly by transmembrane TNF. Upon TNF binding to TNFR, the adaptor molecule TRADD binds to the death domain of the receptor. TRADD acts as a platform adaptor that can recruit downstream proteins [61]. Chemical cross-linking of TNFR molecules expressed on the cell surface demonstrated that the receptors spontaneously form a homotrimeric structure prior to ligand binding [62].

Other cell-surface receptors

Toll-like receptors (TLRs) recognize structural and sequence variations between host and microbial nucleic acids in immune cells. TLR9 is activated by DNA that is rich in unmethylated CpG motifs, such as microbial DNA, in the endosome. This results in production of inflammatory cytokines and interferons that lead to adaptive immunity. FRET analysis of TLR9 in living cells demonstrated the existence of preformed TLR9 homodimers. TLR9 activation is regulated by conformational changes specifically induced by foreign DNA [63].

Natriuretic peptides play key roles in cardiovascular homeostasis, and their cellular effects are mediated via the transmembrane natriuretic peptide receptor A (NPRA). NPRA, which consists of an extracellular ligand-binding domain, a single TMD, a kinase-homology domain, and a guanylyl cyclase domain, produces cGMP upon ligand binding. Crystallographic analysis demonstrated that the ECD of NPRA is homodimeric in the absence of bound ligand [64]. Domain-swapping analysis and site-directed mutagenesis demonstrated that the transmembrane guanylyl cyclase GCY-14, which senses extracellular alkalinity in the nematode, *Caenorhabditis elegans*, also has a homodimeric structure [65]. Histidine residues in the GCY-14 ECD are essential for extracellular alkalinity sensing. The transmembrane protein LINGO-1 is a negative regulator in the nervous system, mainly affecting axonal regeneration, neuronal survival, oligodendrocyte differentiation, and myelination. Co-immunoprecipitation and BRET saturation analyses have shown that LINGO-1 is homodimeric on the cell surface [66]. It has long been known that bacterial chemoreceptors, such as Tar and Tsr, exist in dimeric form on the cell surface [67, 68]. The same is true for the *Escherichia coli* extracellular osmolarity sensor EnvZ [69].

How are preformed dimeric receptors activated by ligand binding?

For increasing numbers of cell-surface receptor dimers, like IR and IGF1R, activation cannot be explained by the ligand-induced dimerization model. Early studies demonstrated that a chimeric receptor with the IR ECD and the EGFR ICD was activated by insulin, and that EGF activated a chimera with the EGFR ECD and the IR ICD [70–72]. Furthermore, a chimeric receptor, consisting of the ligand-binding ECD of bacterial Tar and the IR ICD, is activated by aspartate, resulting in phosphorylation of the intracellular IR moiety [73]. These studies suggest that diverse cell-surface receptors may be regulated through similar molecular mechanisms.

“Rotation model” for transmembrane signaling by Tar and EGFR

Bacterial chemotaxis is a model system for signal transduction, and the chemoreceptor Tar is one of the best-characterized cell-surface receptors. A proposed “rotation model” for transmembrane signaling by the Tar dimer indicates that ligand binding to the Tar ECDs is likely to restrict rotation of the TMDs at specific positions about their long axes [74]. The model predicts that Tar molecules with and without bound aspartate have similar structures, since bound aspartate stabilizes the most stable structure of the apo-receptor. Indeed, crystal structural analysis demonstrated that the membrane proximal region of the Tar ECD with bound aspartate translates $\sim 1 \text{ \AA}$ downward or toward the cytoplasm compared to its position without bound ligand [75]. A similar subtle ($\sim 1 \text{ \AA}$) movement of the TMD was also detected by electron paramagnetic resonance spectroscopy analysis of spin-labeled receptors, with and without bound aspartate [76]. These results can be interpreted to indicate that binding of aspartate further stabilizes the most stable structure of apo-Tar in the absence of bound aspartate, although the “piston model” [75, 76] among others [77, 78] has also been proposed. Furthermore, the “rotation model” also predicts that TMD of attractant-bound and repellent-bound forms differ rotationally by an angle of $\sim 50^\circ$. These restricted rotations of the TMDs may in turn restrict rotation of the HAMP domains in the cytoplasm in order to regulate activity of the histidine kinase CheA, which physically interacts with Tar with help of the adaptor, CheW. This model is consistent with recent results showing axial helix rotations of the Tsr cytoplasmic domains during transmembrane signaling [79].

A similar “rotation model” has also been proposed for activation of the EGFR, in which EGF binding to its flexible ECDs induces conformational changes of the domains to form a stable dimeric structure. Extracellular conformational changes induce a rotation ($\sim 140^\circ$ parallel to the plane of the plasma membrane) of the TMDs about their long axes, which in turn dissociate the inactive, symmetric kinase dimer in

the cytosol, followed by rearranging of dimeric kinase domains to form active asymmetric structures [9, 10, 18]. Consistently, computational analysis of the conformation space of the ErbB2 TMD homodimer has supported a molecular mechanism for rotation-coupled receptor activation, in which the two stable conformations of the TMD correspond to the active and inactive states of the receptor [80].

IR family may also be activated by its TMD rotation

Crystal structures of IR ECDs without ligand [81, 82] and a fragment of the IR ECD bound to insulin [83] have been determined. In the absence of ligand, the ECDs form a symmetric, antiparallel dimer shaped like a folded-over “Λ.” The C-terminal half of the ECD consists of three contiguous, fibronectin type III (FnIII) domains, which are followed by the TMD. Bioinformatics analysis indicates that only a slight “rotation” of the last two FnIII domains is required to align the proposed binding sites of IR to insulin [7, 84]. This subtle “rotation” of the extracellular juxtamembrane region and TMD during signaling is compatible with results of a small-angle X-ray scattering study of IGF1 binding to the soluble IGF1R ECD, wherein very little change in the radius of gyration was observed in the ECD upon binding of IGF1 [85].

An alternative model has recently been proposed based on FRET and mutagenesis studies, in which the IGF1R ECD maintains an auto-inhibited state with the TMDs held apart. Ligand binding releases the constraint, allowing association of TMDs and kinase domains for trans-autophosphorylation [86]. Deletion of the extracellular N-terminal L1 domain of IGF1R resulted in constitutive activity of IGF1R. This is reminiscent of the EGFRvIII mutant, in which an extracellular, N-terminal ligand-binding domain is deleted [87]. EGFRvIII exists in dimeric form and is constitutively active in the absence of bound ligand. In both EGFR and IGF1R, the extracellular ligand-binding domains seem to play a role in keeping the intracellular kinase inactive prior to ligand binding. Deletion of their ligand-binding domains may induce or allow a rotation of their

transmembrane domains for activation of the kinases. Indeed, cysteine residues artificially introduced into the extracellular juxtamembrane region of IGF1R formed disulfide bridges in the absence of bound IGF1, and the cross-linked receptors were autophosphorylated as efficiently as in the presence of bound ligand. These results indicate that the juxtamembrane regions (hence the transmembrane domains) exist in close enough proximity to spontaneously form disulfide bridges in the absence of ligand. This is consistent with the dimeric structure of unactivated IGF1R kinase domains, determined by crystallography, in which two monomers are arranged such that their ATP binding clefts face each other. The ordered N-terminus of one monomer approaches the proximal part of the activation loop, the ATP binding pocket, and the catalytic loop of the other monomer [88].

Another model recently proposed for IR activation is based on results in which IR TMD peptides supplied extracellularly, stimulated a dose-dependent increase in IR tyrosine phosphorylation in living cells. This result was interpreted as indicating that TMD peptides specifically interact with an inactive form of IR TMD dimers, resulting in dissociation of the TMD dimer to activate the receptor [89]. As discussed above, the TMD peptides interact with an inactive form of IR TMD dimers, and may induce or allow a rotation of the IR TMD about its long axis for rearrangement and activation of the IR kinase dimers.

Homodimeric EpoR TMDs rotate during signaling

Crystallographic structural analysis of the EpoR ECD in the presence and absence of bound ligand suggest that the receptor may exist in dimeric form prior to ligand binding [51]. From a subsequent fluorescent study based on dimerization-induced complementation of designed fragments of the murine enzyme dihydrofolate reductase, an allosteric mechanism of EpoR activation was proposed in which ligand-induced reorganization of the dimer brings the intracellular domains into closer proximity, allowing associated JAK2s to come into contact and autophosphorylate [90]. However, the mechanism is now explained differently in which

activation of dimeric EpoR by erythropoietin binding is achieved by rotationally reorienting the receptor TMD and connected cytosolic domains, through random mutagenesis of the TMD, which was followed by cysteine-scanning mutagenesis of the receptor juxtamembrane and TMDs [91]. Analysis of chimeric receptors of the EpoR, in which its ECD was replaced with a dimeric, coiled coil, has also demonstrated three rotationally related conformations (active, inactive, and partially active) of EpoR TMD dimers [92]. When the engineered EpoR fusion protein was constrained in seven possible orientations, three dimeric TMD orientations corresponding to fully active, partially active, and inactive receptors were identified by measuring activity of JAK2, STAT, and MAP kinases in the cytosol. Average molecular structures for active and inactive orientations differ by a rotation of $\sim 100^\circ$. Ligand-induced rotations of EpoR TMDs may induce flexibility of the receptor's ICDs, and may rearrange the JAK2 kinase dimer for its autophosphorylation.

TMD rotations during signaling by GHR, TpoR, NPRA, Eph, and VEGFR

Within the dimeric GHR, subunit rotations ($\sim 40^\circ$ clockwise) have been suggested as the activation mechanism, using FRET, BRET, and co-immunoprecipitation [93, 94]. Once GH is removed from the hormone-bound receptor complex, consistently, counter-clockwise rotations of $\sim 45^\circ$ of the two subunits relative to each other has been observed in atomistic molecular dynamics simulation [95]. Three different, rotationally related conformations of TpoR TMD dimers, possibly corresponding to specific states (active, inactive, and partially active) of the full-length receptor have been discovered with a combination of cysteine cross-linking, alanine-scanning mutagenesis, and computational simulations [53]. The active interface between dimerized TMDs differs from the inactive interface by a rotation of $\sim 100^\circ$. Similarly, a transmembrane rotation of 40° that leads to constitutive activation of NPRA has been elucidated by sequentially replacing nine residues with cysteine and by introducing one to

five alanine residues into the receptor transmembrane α -helix [96].

Structural analysis of EphA2 TMD dimers in lipid bicelles using solution NMR found that there are two states, left-handed, parallel-packed, and right-handed, dimeric structures, suggesting a rotation-coupled (60° , average) activation mechanism during EphA2 signaling [41]. Indeed, site-directed mutagenesis of TMD of full-length EphA2 suggests that the TMD domains interact in two different ways, corresponding to inactive and active receptor states, respectively, as a mechanism underlying EphA2 signal transduction [97]. When EphA1 TMD dimers in lipid bilayers were analyzed with a multiscale approach, combining coarse-grain and atomistic molecular dynamics simulations, it was found that the interaction of transmembrane helices in EphA1 dimers may be intrinsically flexible enough to accommodate two states involving helix rotations about their long axes [98]. Similarly, NMR revealed that vascular endothelial growth factor receptor 2 (VEGFR2) TMD

helices in activated constructs were rotated by 180° relative to the interface of the wild-type conformation [99].

TMD rotations as a common mechanism underlying transmembrane signaling by cell-surface receptors?

As described above, transmembrane signaling by a variety of cell-surface receptors may be regulated by bound ligand through a common mechanism, in which ligand binding to receptor ECDs induces their TMD rotation, thereby regulating ICD activity (the “rotation model,” Fig. 1). Conformational changes of ECD dimers induced by ligand binding are likely to induce a rotation of the receptor TMDs, resulting in rearrangement of the ICDs. As observed in EGFR [9, 10], structures of receptor ECDs and ICDs are flexible and less flexible, respectively, prior to ligand binding. Ligand binding is likely to stabilize the flexible ECDs. The resulting

conformational changes of the ECDs induce a rotation of the TMDs, which makes the ICDs less stable and rearranges the domains. TMD rotations occur together with changes in interhelical crossing angles and distances, as observed in an NMR study of the EphA2 TMD [41]. In addition to TMD rotations, indeed, such interhelical crossing angles and distances are also crucial for activation of GHR and EGFR [100, 101]. During transmembrane signaling mediated by cell-surface receptors, TMD rotations would be energetically favorable in comparison to TMD’s lateral movement against the lipid bilayer barrier, proposed by the ligand-induced dimerization model. The “rotation model” may also explain not only outside-in but also inside-out signaling mechanisms. During inside-out signaling, conformational changes of receptor ICDs induced by cytoplasmic factor(s) may stabilize ICD flexibility, and may induce TMD rotations opposite in direction to those of outside-in signaling, as observed in atomistic molecular dynamics simulation of GHR [95]. This counter rotation of TMDs may induce flexibility of ECDs, and may release ligand from the domains.

To test the model, it is necessary to analyze structures of full-length receptors in the presence and absence of bound ligand, since the receptor extracellular juxtamembrane regions, TMD and ICD, seem to play crucial roles in dimer formation [10, 18]. Furthermore, the dimeric receptor structures are very unstable outside of the membrane. Therefore, structures should be determined in the membrane, intact or artificial. Cryo-electron tomography [102] may be suited for experiments in cases in which large conformational changes, like those observed in EGFR, are expected. Various conformational structures of ECD dimers with a relatively stable ICD dimer may be observed prior to ligand binding. In the presence of bound ligand, in contrast, a relatively stable structure of ECD dimers with various conformational variables of ICD dimers may be observed.

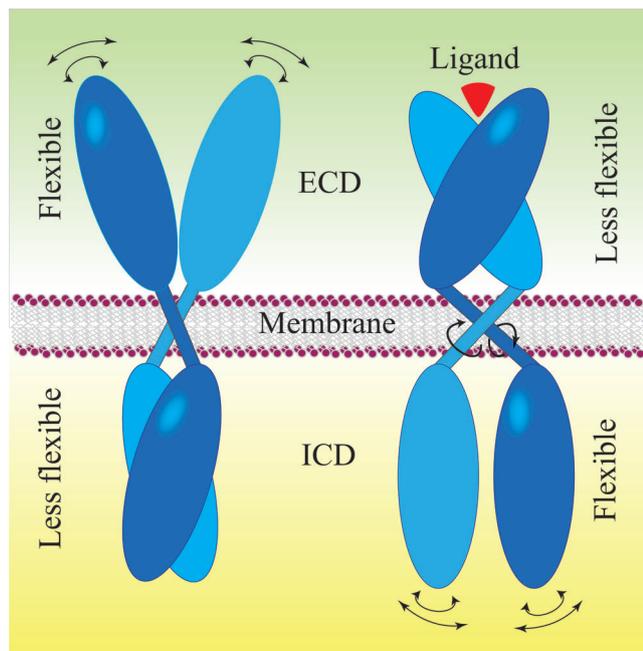


Figure 1. “Rotation model” of transmembrane signaling mediated by cell-surface receptors. Prior to ligand binding, receptors exist in dimeric form on the cell surface. The ICD dimer has a relatively stable structure while the ligand-binding ECD dimer has a rotationally flexible structure. Ligand binding stabilizes the flexible ECDs and induces conformational changes of the domains. This extracellular conformational change in turn induces or allows a rotation of the TMDs, which rearranges the ICDs, making them flexible for activation and/or interaction with other cytoplasmic proteins. Rotation of TMDs occurs together with changes in interhelical crossing angles and distances.

Conclusions and outlook

It has traditionally been thought that transmembrane, cell-surface receptors are activated by ligand-induced

dimerization of the receptors. As we have seen, however, many receptors exist in constitutively dimeric form prior to ligand binding. The number of preformed dimeric receptors is likely to increase in the near future. If receptor tyrosine kinases and cytokine receptors exist as monomers, it would be harmful to cells because random collisions of receptor monomers on the cell surface could accidentally activate them in the absence of ligand. Under evolutionary pressure, cell-surface expression of receptor monomers might have disappeared from transmembrane signaling processes. There is also growing evidence that a common molecular mechanism, TMD rotations about their long axes, regulates activity of dimeric receptors. This mechanism nicely explains not only outside-in transmembrane signaling, but also inside-out signaling. TMD rotations for regulation of receptors present new opportunities for design of antagonists and agonists as pharmaceuticals.

Acknowledgments

The author is grateful to Charlotte Fournier and Takashi Yonetani for their invaluable comments on the paper, and Hitomi Ohtaki for assisting in drawing the figure. I also thank my laboratory members for their critical reading of the paper. This work was partly supported by funding to the Information Processing Biology Unit from the Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan.

The author has declared no conflicts of interest.

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