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Editorial

Transmembrane signaling: A multiplex problem with converging solutions

Transmembrane receptors transmit biological signals across the membrane and are essential communicators of information between cells and between cells and the environment. They have an overall architecture that spans the cell membrane, consisting of extracellular, transmembrane and cytoplasmic domains. They have the general function of converting physical energy of extracellular ligand binding to intracellular chemical reactions. Although these general properties have been known for many years, the physical and structural basis of how ligand binding triggers and amplifies transmembrane signals remains largely mysterious for most receptors. These questions brought a group of scientists from around the world to Suzhou, the beautiful garden city of China, to exchange ideas in a Cold Spring Harbor Asia meeting focused almost exclusively on the mechanism of transmembrane signaling, organized by us together with Tom Blundell of University of Cambridge and Michael Sheetz of the National University of Singapore and Columbia University. The meeting turned out to be both useful and inspiring, featuring new structures and functional data, new technologies for investigating transmembrane receptors, as well as a variety of concepts that Nature might employ to achieve transmembrane signaling. Published in this themed volume are only some but nevertheless a good representative selection of those presentations featured at the meeting.

The meeting began with a keynote speech by Tom Blundell on structural biology of receptor tyrosyl kinase clustering, activation and modulation, followed by several beautiful crystallographic works on receptor ligand recognition, which included discussion of ligand binding of growth factor receptors as well as cytoplasmic signaling components of these receptors. Presented in this volume (Blaszczyk et al., *PBML*, 2015; 103–111), Blundell and his colleagues first describe two different modes of ligand binding to the ectodomain exemplified by insulin and glucagon receptors, one through preformed globular domain and the other through folding-upon-binding mechanism. They then focus their discussion on two receptor tyrosyl kinases: the fibroblast growth factor receptor (FGFR) and the MET receptor. Through a series of structural analyses, they suggest that simple dimerization models might occur opportunistically, giving rise to noise in the crowded cellular environment. They consider the cooperative clustering of receptor tyrosyl kinases observed in these multiprotein systems to be selectively advantageous to signal transduction above the noise level. They further ask whether this might be a more general scenario for high signal-to-noise signal transduction for many multi-component transmembrane receptor-signaling systems. The authors thus provide a conceptually new argument for more complex transmembrane signaling assemblies.

Rob Meijers and Jia-huai Wang summarize the structural biology of an important axon guidance receptor, DCC (deleted in colorectal cancer) (Finci, L et al., *PBML*, 2015; 153–160). The unique feature of this single-pass transmembrane protein is that it comprises a 10-domain ecto-fragment, but a large (about 350 residues) intrinsically disordered cytoplasmic tail, responsible for signaling. The most interesting part of the review focuses on how the high-profiled guidance cue, netrin-1 binds to DCC. Two complementary structural and functional studies with somewhat different constructs have been described. One by the authors themselves features two DCC receptor-binding sites on one netrin-1 ligand. One site is DCC-specific, whereas the other is relatively generic allowing the other receptor to bind. Combined with functional assays, a possible netrin-1 bi-functionality signaling model has been proposed. The other structure by Tessier-Lavigne and Nicolov suggests the clustering mechanism of DCC receptor by ligand.

Yvonne Jones presents structural and biophysical studies of a unique class of single-pass transmembrane receptors (Zebisch, M. and Jones, Y. *PBML*. 2015; 112–118). Instead of having a typical "signaling-type" cytoplasmic domain, these receptors (ZNRF3 and RNF43) bear E3 ubiquitin ligase function in their cytoplasmic tail. Based on a number of structures determined in their laboratory, the authors describe that this unusual combination of a receptor-like ligand-binding ecto-domain with an intracellular E3 ligase activity reveals a ligand-binding mechanism at atomic resolution. They discuss how these receptors play important roles in the Wnt signaling pathway by serving as the clearance machinery for the Frizzled membrane receptors. Here the specific ligand recognition of the ectodomain is similar to that of classic signaling receptors; the high order oligomerization and clustering are also similar, but the cytoplasmic function differs. It provides a novel mechanism for extracellular control of ubiquitin ligase activity used in many cellular processes.

Moving away from the cell surface into the cytoplasmic region, Xuewu Zhang gives a complementary review of possible cytoplasmic signaling mechanism of plexin, the major cellular receptor for semaphorin (Pascoe, H.G., et al., *PBML*. 2015; 161–168). The semaphorin/plexin signaling plays critical roles in axon guidance, angiogenesis and many other cellular processes. Activated by semaphorin, the GTPase activating protein (GAP) domain in plexin's cytoplasmic region tranduces the downstream signaling pathway. Most interestingly, based on structural comparison, the authors discuss how the semaphorin-ligand dimer binding to two plexin receptors triggers dimerization-driven activation of plexin GAP activity. The cytoplasmic region of plexin changes its conformation from the "closed" autoinhibitory monomeric state to a more "open"

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activated dimeric state, which may further lead to clustering on the cell surface, giving rise to another level of plexin signaling regulation.

Compared to the well-structured extracellular ligand-binding or intracellular-kinase domains, the transmembrane and membrane-associated regions of receptors have historically occupied a major "blind spot" in structural biology, due to their intrinsic properties that resist crystallization. In the past decade, there has been increasing recognition that the transmembrane helices of receptors are not merely inert anchors but in fact play essential roles in the oligomerization of receptor subunits within the membrane. The modes of association between the transmembrane helices directly link the conformational state of the extracellular domains to that of the juxtamembrane regions and the cytoplasmic-signaling motifs. Chenqi Xu discusses membrane interaction of the membrane-proximal regions of the T cell receptor (TCR) complex that contain the ITAM signaling motif (Wu, W., et al., *PBML*, 2015; 130–138). Specifically, he describes how acidic phospholipids regulate antigen-induced tyrosine phosphorylation of TCR through ionic protein-lipid interaction while illustrating the versatile use of NMR and bicelles in characterizing the structural and dynamic properties of membrane-proximal regions of receptors.

The B cell receptor (BCR) complex is simpler than the TCR, but the overall design of having receptor modules for ligand recognition and signaling modules for phosphorylation is similar. The receptor module of BCR is a membrane-anchored immunoglobulin M (IgM), which is a homodimer responsible for antigen binding. There are two signaling chains, Igα and Igβ, each containing an extracellular domain that associates with the IgM, a transmembrane domain, and a cytoplasmic tail containing the ITAM signaling motif. While the ITAM of CD3e of TCR is largely bound to the membrane via interactions with acidic phospholipids, it was not known whether the ITAMs of BCR have the similar membrane association properties. Liu and colleagues describe the membrane-bound properties of the BCR ITAM and their implication for understanding the enhanced activation of the IgG-switched memory B cells upon BCR engagement with antigen (Chen, X., et al., *PMBL*, 2015; 89–94).

For the growth factor and immune receptors discussed above, ligand binding induces dimerization or higher order clustering of receptors, and the ligand-induced aggregation or clustering is important for achieving significant phosphorylation activities on the cytoplasmic side of the membrane. The transmembrane signaling of the two-component regulatory systems in bacteria, however, may use different mechanisms. The two-component systems generally comprise a sensor component that receives an environmental signal and transmits it, via phosphorelay, to an intracellular response regulator protein. The sensor component is a transmembrane receptor and has a domain organization that is similar to the receptor tyrosine kinases, except that the cytoplasmic kinase domain is a histidine kinase. Linda Kenney describes the signaling mechanism of the fascinating EnvZ two-component system that senses osmolality (Foo YH., et al., *PBML*, 2015; 119–129). Her lab found that the membrane-proximal four-helix bundle on the cytoplasm flanking the histidine kinase appears to undergo conformational change associated with osmosensing. The two-component sensors can sense a large variety of environmental factors from nutrients and antibiotics to temperatures and pH. Their extracellular domains can be as large as several hundred residues or as small as nine residues in the case of SaeS, a histidine kinase receptor that regulates the transcription of extracellular toxins to resist neutrophils. Future efforts to investigate the structural and physical basis of sensing by the receptors of the two-component systems are expected to reveal many more solutions that Nature employs to transmit information across biological membrane.

Although the mechanisms of most transmembrane receptors are not understood, it is already clear that Nature has evolved diverse means of transmitting the signal of ligand binding to the extracellular domain to phosphorylation of the intracellular signaling domains, and these could involve conformational change of the receptor, defined receptor oligomerization, and less well-defined receptor clustering. A multi-disciplinary approach is thus needed to investigate thoroughly the diverse set of mechanisms. Fang and colleagues describe their recent work on using single-molecule fluorescence microscopy to probe the dynamics of two growth factor receptors, the transforming growth factor receptor (TGFR) and epidermal growth factor receptor (EGFR) (Sun Y., et al., *PBML*. 2015; 95–102). The method enables the characterization of receptor stoichiometry, monomer-dimer interconversion kinetics, effects of microenvironment on receptor membrane diffusion, and intracellular transportation under different signaling conditions. In another development, Needham and colleagues describe a method named "fluorophore localization imaging with photobleaching" (FLImP), which uses single molecule localization and single-step photobleaching to determine the separation of two fluorophores with a resolution of 7 nm or better. Recent application of the method on EGFR has shown that the intracellular domain of the receptor is not required in the basal state for the receptor to form ordered inactive oligomers in the plasma membrane (Zanetti-Domingues, LC. et al., *PBML*. 2015; 139–152).

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