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Review

Conformational transitions and interactions underlying the function of membrane embedded receptor protein kinases☆

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ABSTRACT

Among membrane receptors, the single-span receptor protein kinases occupy a broad but specific functional niche determined by distinctive features of the underlying transmembrane signaling mechanisms that are briefly overviewed on the basis of some of the most representative examples, followed by a more detailed discussion of several hierarchical levels of organization and interactions involved. All these levels, including single-molecule interactions (e.g., dimerization, liganding, chemical modifications), local processes (e.g. lipid membrane perturbations, cytoskeletal interactions), and larger scale phenomena (e.g., effects of membrane surface shape or electrochemical potential gradients) appear to be closely integrated to achieve the observed diversity of the receptor functioning. Different species of receptor protein kinases meet their specific functional demands through different structural features defining their responses to stimulation, but certain common patterns exist. Signaling by receptor protein kinases is typically associated with the receptor dimerization and clustering, ligand-induced rearrangements of receptor domains through allosteric conformational transitions with involvement of lipids, release of the sequestered lipids, restriction of receptor diffusion, cytoskeleton and membrane shape remodeling. Understanding of complexity and continuity of the signaling processes can help identifying currently neglected opportunities for influencing the receptor signaling with potential therapeutic implications. This article is part of a Special Issue entitled: Interactions between membrane receptors in cellular membranes edited by Kalina Hristova.

Contents

Abbreviations: RTK, receptor tyrosine kinase; TKL, tyrosine kinase-like receptor; GPI-AP, glycosylphosphatidylinositol-anchored protein; SH2, Scr homology 2 domain; TMD, transmembrane domain; ECD, extracellular domain or ectodomain; ICD, intracellular domain; JM, juxtamembrane; IDP, intrinsically disordered proteins; PA, phosphatidic acid; PS, phosphotidylserine; PIP, phosphatidylinositol-phosphate; Ld, liquid disordered; Lo, liquid-ordered.

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1. Distinguishing traits of single-span receptor protein kinases and implications for possible signaling mechanisms

Single-span membrane proteins (also known as bitopic proteins) form the absolute majority of the integral membrane proteins, constituting nearly 50% of the entire protein population of a crowded membrane, somewhat surprisingly followed by large polytopic proteins, such as 7-span G-protein–coupled receptors, accounting for about 13% [1]. Many of the G-protein–coupled receptors are signaling proteins, in which the intracellular response is understandably coupled to extracellular events (usually ligand binding) by a complex of protein-protein interactions (though importance of non-protein players in signal transduction is currently emerging). Due to the recent progress in structural methods, the investigations of the G-protein–coupled receptors form a very dynamically evolving area of structural biology and pharmacology, as extensively reviewed in multiple excellent publications. Less predictably, a large proportion of the abundant single-span proteins is also cell signaling receptors, such as receptor protein kinases, the mechanisms of coupling between the extracellular and intracellular events for which are but vaguely understood and appear to involve more diversity, both in terms of the mechanism itself and of the signaling process participants. Generally speaking, these two classes of receptor architectures correspond to different temporal domains and classes of substrates, the single-span receptor protein kinases being used when a more long-term sustained and/or differentiated response is needed for slow cellular processes, such as rearrangement of cellular matrix, cell proliferation and differentiation, but usually requiring larger ligands to achieve deterministic signal transduction across the membrane. The versatility of ligands interacting with the extracellular domains (ECD or ectodomains) and the cross-talk between different membrane receptor species that can bind overlapping subsets of ligands and form heteromers with others are translated into complexity and versatility of the events initiated on the intracellular side. This complex mosaic is now extensively studied, with many biologically relevant implications already established at some level of details [2–5].

Another broad range of issues is related to the question how exactly does a ligand-induced rearrangement of a usually bulky water-soluble ECD (usually containing multiple subdomains) translate into fairly complex and deterministic response of the intracellular domain (ICD) (typically consisting of two subdomains) with just a single helical transmembrane domain (TMD) linked *via* highly flexible juxtamembrane (JM) regions. In a sense, a single transmembrane helix implies certain looseness of coupling between the extracellular and intracellular events. Moreover, unlike the soluble domains, the TMD sequences are generally not highly conserved, and even related protein kinases apparently sharing common signaling mechanisms often have diverse TMDs. Only certain, presumably functionally relevant, common individual residues and characteristic motifs (e.g., dimerization, lipid recognition etc.) can usually be identified [6–14]. The apparent contradiction of the allegedly loose coupling and deterministic signaling response is yet unresolved, partly because of the difficulty to obtain structural information on full-size receptors due to a high degree of heterogeneity, owing to the presence of large mobile water-soluble domains connected *via* flexible linkers, and TMDs requiring lipidic environment. In principle, signaling by a monomeric single-span receptor is possible, but in the absolute majority of cases, dimers or higher oligomers are formed to deliver the biological function. Several general ideas have been suggested to account for the signaling mechanism and are now commonly acknowledged, starting from the most straightforward idea of ligand-induced dimerization, where active state of the receptor is achieved by formation of a dimer, e.g., enabling some kind of interaction between the intracellular domains of individual monomers [15,16]. The signaling process via such a mechanism would be, however, diffusion limited, and therefore in many cases inactive receptor dimers (oligomer) were shown to preform, allowing better response time [15–19]. Obviously, the ligand-induced dimerization signaling mechanism is not a viable option in this case, and more sophisticated hypotheses were suggested including ligand-induced rotation or other (twisting, tilting, pistoning, kinking) motions and partial folding/unfolding of the TMD helices, or string-puppet-like mechanistic effects of the ECD rearrangements on the TMD–ICD configuration [17,19–22]. Common to all these mechanistic interpretations of the events behind the signal transduction is the view of the receptor as a soloist in the spotlight surrounded by the dumb bulk of cytosole, cellular membrane and extracellular luminal liquid. Though effective to explain many isolated functional observations and structural properties of the receptor, none of these mechanisms appears to suffice to accommodate the growing amount of physiological, biochemical, biophysical and structural data. In the light of the new evidence, it appears increasingly likely that it is the retinue that makes the king, and that every piece of the alleged "environment" has an active role in the signal transduction, and their cumulative contribution may well be definitive. This is not limited to the different proteins participating in signaling (directly or *via* regulatory mechanisms, e.g. phosphorylation or glycosylation), but rather includes the entire ensemble: the surrounding bulk lipids and specific lipid or glycolipid specimens, elements of cytoskeleton and extracellular matrix, and even ubiquitous water-soluble components, e.g. inorganic cations or protons (pH). It is the mechanism underlying signaling by single-span receptor proteins that we will focus on in this review, with the emphasis on receptor protein kinases, for which autophosphorylation and transphosphorylation of multiple targets and effectors are the key signaling event.

Any attempt of classification of receptor protein kinases reveals certain commonalities in their structural and spatial organization, potentially informative of the structure-function relations within the superfamily. To wit, whereas the ECDs of representatives of each subfamily are demonstrably unlike each other, all of them share a single helical TMD flanked on each side by relatively long (upwards of 30–40 residues), at least partly amphiphilic and flexible JM regions. While a single transmembrane helix can appear to be simply the most economical way to link the intracellular and extracellular parts of the receptor, as is necessary for any specific signal transduction mechanism imaginable (though it also understates the TMD role), commonality of certain specific properties of the JM linker regions across the superfamily clearly suggests they play a functional role. The cytoplasmic JM and C-terminal tail (superseding the kinase domain) regions have distinct resemblance to intrinsically disordered proteins (IDP) [23], being capable of easily changing conformational states and interacting transiently with the membrane surface or accommodating to aqueous environment and interacting with the cytoplasmic participants of the signaling process [24–28]. These dual properties can be affected by phosphorylation, and accordingly, both regions often have sites for regulatory phosphorylation and serve as signal-transduction phosphorylation targets. Formation of the dimeric or oligomeric signaling-competent complexes in most cases precedes ligand binding, with the monomer-dimer (oligomer) equilibrium usually poised towards the dimeric (oligomeric) forms. However, diverse pathways of achieving active dimeric or oligomeric signaling complex are used by different individual representative of single-span receptor proteins. The events constituting signal transfer across the membrane can also be different. Although in this review we mostly discuss receptor tyrosine kinases (RTK) or tyrosine kinase-like (TKL) receptors, for which the downstream signaling cascade is initiated by phosphorylation of tyrosine, serine or threonine residues [2,29,30], cleavage of the cytoplasmic part of the receptor by membrane-embedded enzymes, such as γ-secretases, is another option, described for certain receptor families [31].

An illustrative example is provided by the ErbB/HER receptors (the human epidermal growth factor receptor (EGFR) subfamily of RTK [2, 32,33]), whose active signaling complex consists of hetero- or homodimers, which were often claimed to be formed *via* ligand-induced dimerization mechanism. However, existence of inactive preformed homo- and/or heterodimers has been explicitly demonstrated, as well as the ability of ligand-binding to activate the pre-dimers [16,

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