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Progress and prospects for structural studies of transmembrane interactions in single-spanning receptors

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Single-spanning receptors are typically active in dimeric or oligomeric forms in which ligand-induced complex formation and/or conformational changes are the key events that transmit information across the cell membrane. This process is often depicted exclusively in terms of extracellular receptor–ligand interactions and their intracellular consequences, but the lipid-embedded α-helical transmembrane domains can also engage in specific intermolecular interactions that play important roles in establishing receptor complex structure and regulating signal propagation through the lipid bilayer. Obtaining high-resolution structural information on these interactions is extremely challenging, and the small number of structures currently available in the protein data bank represents only about a dozen unique receptors. In this review, we highlight new structures that provide novel insights into receptor tyrosine kinase and death receptor function and discuss the implications of recent successes in the application of X-ray crystallographic techniques to determine the structures of receptor transmembrane complexes in lipid bilayers.

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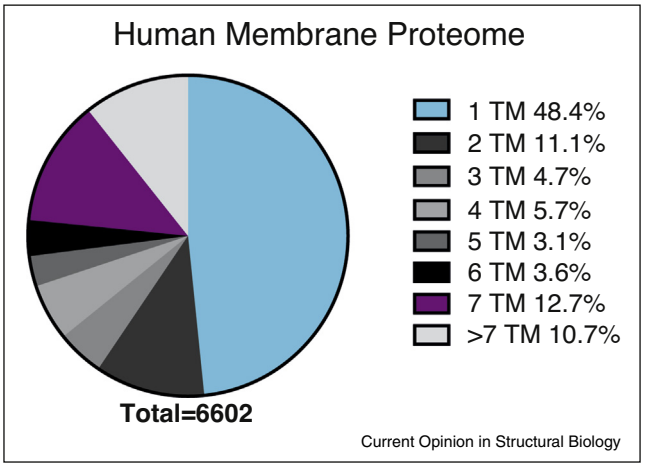
Introduction

Cells communicate with one another and respond to environmental cues primarily through the actions of cell-surface receptors that convert extracellular molecular encounters into intracellular biochemical signals. How this conversion takes place across the plasma membrane is a fundamental biological problem for which powerful mechanistic insights have been gained from atomic-scale structural studies. Receptors comprising single-spanning (bitopic) membrane proteins, or assemblies thereof, present their own unique set of technical challenges for structural biologists, and our understanding of how extracellular ligand binding and intracellular signaling are coupled across the membrane is therefore not well developed in most systems. High-resolution structural characterization of intact single-spanning receptors has not yet proven feasible using currently available techniques, and most studies have therefore focused on truncated extracellular and intracellular water-soluble domains. However, we now understand that the α-helical transmembrane (TM) domains frequently make crucial contributions to the assembly of oligomeric signaling complexes and the conformational changes involved in receptor activation [1–3]. Understanding the structural features governing these functions is therefore an important priority in receptor biology.

Nearly half of the proteins in the human membrane proteome are predicted to contain a single α-helical membrane-spanning domain (Figure 1) [4]. A large proportion of these are classified as receptors, and many have genetic and/or biochemical evidence that TM interactions are involved in establishing their higher-order structures and in executing their functions [2]. However, structural studies of single-pass receptor TM interactions have seen relatively few successes, and these have been achieved almost exclusively using solution NMR techniques. The methodology involved in working with TM peptide complexes remains challenging, generally requiring specialized production and purification approaches, careful screening of membrane mimetics, sophisticated isotopic labeling strategies and advanced spectroscopic techniques. Consequently, the available structures have come from just a handful of laboratories specializing in these methods. Nonetheless, the available structures paint a highly informative picture of some of the ways in which single α-helical TM domains can interact to influence receptor structure and function: well known examples include the erythrocyte surface protein glycophorin A [5,6], the immunoreceptor-associated signaling modules DAP12 [7] and ζ chain [8], integrin $\alpha_{iib}\beta_3$ [9] and several receptor tyrosine kinases (RTKs) of the ErbB [10–13] and Eph families [14,15]. These structures and the mechanistic insights they provide have been extensively reviewed [1,2,16,17], and they are discussed here only in the context of more recent advances. In this short review, we highlight several studies published in the past three years that provide new insights for long-studied receptor systems, new TM structures for a previously unrepresented receptor

Figure 1



Topological analysis of the human membrane proteome. Single-spanning transmembrane proteins constitute the largest topological class of membrane proteins in the human membrane proteome with a predicted total relative abundance of 48%. TM prediction data were taken from Ref. [4].

family and new methodological advances for structural analysis of single-pass receptor TM interactions using X-ray crystallography.

New structural insights into RTK activation

The receptor tyrosine kinases (RTKs) comprise several sub-families of receptors controlling cell cycle, proliferation, differentiation and migration and are expressed in many cell types [18]. These receptors combine extracellular ligand-sensing and intracellular kinase activities in a single polypeptide that spans the membrane through one α-helical TM domain. One of the most thoroughly studied RTK sub-families is the epidermal growth factor receptor (EGFR) family, comprising EGFR, EGFR2, EGFR3 and EGFR4, which are also referred to as ErbB(1-4) and HER(1-4). Structural studies of isolated domains of EGFR in various states have generated a view of receptor activation in which binding of EGF to its receptor causes a conformational change resulting in formation of a symmetric dimer interface between extracellular domains and an asymmetric dimer of cytosolic kinase domains, wherein the 'receiver' kinase is activated by the 'activator' kinase through an allosteric mechanism [19]. On the basis of structural studies of TM peptides derived from other family members [10–12], the active EGFR dimer is also believed to contain a specific TM interface that acts as a conformational link between extracellular and intracellular events. The TM and kinase domains are connected by a ~40 amino acid region of the receptor referred to as the juxtamembrane (JM) segment. While the analogous region in many RTKs is autoinhibitory [20], the JM segment in EGFR supports kinase activation: a pair of studies published in 2009 [21,22] showed that the kinase-proximal half of the JM segment (JM-B) contacts the kinase domains directly and acts as a latch to stabilize the asymmetric kinase dimer. The membrane-proximal half (JM-A), on the other hand, was proposed to form a short anti-parallel helix dimer that properly positions the kinase:JM-B interactions [21]. The relationship between the TM and JM structures, and how they differ between active and inactive receptors, have remained important open questions.

Structures of membrane-associated domains in the activated EGF receptor

In 2013, Endres *et al.* [23••] determined the solution NMR structure of an EGFR TM–JM fragment in lipid bicelles that provided key insights into the conformational coupling between these regions (Figure 2). As seen in previous NMR structures of the closely related ErbB2 [11], ErbB1/2 [12] and ErbB4 [10] TM dimers, the TM domains of EGFR adopt a right-handed dimer interface through N-terminal glycophorin A (GpA)-like small amino acid motifs (small-xxx-small, where 'small' is glycine, serine or alanine and x is any amino acid) (Figure 2a,b) [23••]. This structure is consistent with molecular dynamics simulations reported in an accompanying paper [24•] and with earlier disulfide cross-linking studies identifying a similar interface in the ligand-bound, full-length EGFR protein [25]. Disruption of this TM interface through simultaneous mutation of four small amino acids (two overlapping GpA-like motifs) to isoleucine impaired the response to EGF in cells [23••], indicating that formation of this TM structure is indeed critical to signaling. Importantly, the geometry of the TM dimer provides ideal spacing at the intracellular membrane face to support the antiparallel coiled-coil structure in the short JM-A sequence [21] (Figure 2b). The JM structure is both dynamic and highly sensitive to the membrane mimetic conditions used [23••,26], consistent with the notion that this segment rearranges during receptor triggering and that the JM region may interact with negatively charged lipids. Combining these data with extensive MD simulations of receptor fragments in lipid bilayers reported by Arkhipov *et al.* [24•], Endres *et al.* propose a model in which EGF binding to the ectodomain causes close juxtaposition of TM domains near the extracellular membrane limit, supporting the N-terminal TM dimer interface and converting the JM sequences from a membrane-bound, non-interacting form to the antiparallel coiled-coil conformation that supports kinase activation [19].

Structures of EGFR-family TM dimers in 'inactive' conformations

Many RTKs have been shown to exist as a distribution of monomers and dimers at the cell surface even in the absence of activating ligands [27,28], raising the question of what prevents kinase activation in these ligand-free dimeric states. Is there a specific conformation of TM