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Unifying Family A GPCR Theories of Activation

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ABSTRACT

Several new pairs of active and inactive GPCR structures have recently been solved enabling detailed structural insight into the activation process, not only of rhodopsin but now also of the β_2 adrenergic, M_2 muscarinic and adenosine A_{2A} receptors. Combined with structural analyses they have enabled us to examine the different recent theories proposed for GPCR activation and show that they are all indeed parts of the same process, and are intrinsically related through their effect on the central hydrophobic core of GPCRs. This new unifying general process of activation is consistent with the identification of known constitutively active mutants and an in-depth conservational analysis of significant residues implicated in the process.

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1. Introduction: GPCR activation and the differing mechanisms

Human G protein-coupled receptors (GPCRs), with at least 800 unique members (Fredriksson et al., 2003), comprise the largest family of cell-surface receptors. They are ubiquitous biological control points of the cell. External signals are translated by this membrane protein family into readable stimuli resulting in precise cell behaviours. Cell growth and differentiation, cardiovascular function, metabolism, immune responses and neurotransmission are examples of physiological responses controlled by GPCRs (Lagerstrom & Schioth, 2008). They are generally considered to be excellent targets for drug discovery. This comes in part from the compelling role that GPCRs play regulating pathophysiology in a diverse set of disease areas. They also represent the largest family of drug targets with about ~30% of the existing drugs currently targeting GPCRs for their beneficial action, and their therapeutic potential might be even larger. Of the 370 non-olfactory GPCRs 59 have been drugged with small molecules (Congreve et al., 2011). Structurally, GPCRs are cell receptors characterized by seven transmembrane helices clustered in the form of a bundle and linked by three intracellular and three extracellular loops. The available GPCR crystal structures define broadly three distinct conformations: (I) an "inactive state" when the receptor is crystallized in complex with an antagonist or inverse agonist, (II) an "agonist-bound state" lacking the G protein or a surrogate for it and (III) a "fully-active state" resulting from a ternary complex composed by the receptor, an agonist and the G protein (or G protein surrogate). These three states are linked by intermediate conformations. They allow distinct structural features resulting from differences in the chemical structure of the bound ligand that in some cases have been suggested to be related to partial agonism activity (Warne & Tate, 2013). The mechanisms that control GPCR ligand binding and receptor activation are highly complex and have until quite recently been hindered by a lack of structural knowledge of active and inactive states. The design of new therapies with a required

Abbreviations: $5HT_{2A}$, serotonin 2A receptor; A_{2A}, adenosine A_{2A} receptor; CA, constitutive activity; CAM, constitutively active mutant; FSHR, follicle stimulating hormone receptor; GPCR, G protein-coupled receptor; H_1, histamine H_1 receptor; HHM, hydrophobic hindering mechanism; LHCGR, luteinizing hormone/choriogonadotropin receptor; M_2, muscarinic M_2 receptor; MC4R, melanocortin receptor 4; $S1P_1$, sphingosine 1-phosphate receptor subtype 1; StaR, stabilised receptor; TM, transmembrane; TSHR, thyroid stimulating hormone receptor; WT, wild type protein; β_2-AR, β_2 Adrenergic receptor.

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activating or inactivating profile could be significantly enhanced with a more complete understanding of how GPCRs operate at a molecular level, so that this information could then be transferred to the ligands in question.

In the absence of an activating ligand GPCRs typically exhibit basal activity, thought to be caused by the thermal excitation of the environment providing enough energy for a sub-population of receptors to obtain an activated state. This basal activity is enhanced upon binding of an agonist, reduced by inverse agonists and unaffected by a neutral antagonist. Basal activity, the ability of GPCRs to activate in the absence of ligands, can be increased by residue specific mutations, producing constitutively active mutants (CAMs). These CAMs are proposed to change the activation energy pathway or landscape of a receptor, enabling the equilibrium of active to inactive states of a GPCR to shift. Thus the analysis of CAMs provides a valuable insight into the specific residues implicated in activation energy pathway for GPCRs. In addition to significant residues being identified from their constitutive activity (CA), residues that are highly conserved are also thought to play an important role in receptor architecture and a probable common activation pathway (Gether & Kobilka, 1998) of GPCRs. It has long been thought that the mechanism of receptor activation resides in the transmembrane helices and is most probably highly conserved (Gether & Kobilka, 1998), what always has been an issue is how this activation switch has been affected by such a wide range of agonists. Agonists come in a great variety of sizes from small amines to peptides and even up to proteins, and their proposed binding sites also differ significantly from deep inside the receptor to the extracellular surface loops (Strader et al., 1994). Not surprisingly a common mechanism of activation for GPCRs has been difficult to find, although with the advent of recent structures we believe this is now possible.

There have been a number of studies on how GPCRs activate and transmit their signals from the extracellular side through to the G protein on the intracellular side, although recently the reports of most interest are from the two groups who have solved the active structures of rhodopsin (Standfuss et al., 2011) and the β_2 Adrenergic receptor (β_2-AR) (Rasmussen et al., 2011a, 2011b). Interestingly whilst both groups have relatively similar structural endpoints of their respective GPCRs, the proposed activation processes to get to the similar endpoints appear remarkably different.

The group that crystallised the active conformation of rhodopsin (Standfuss et al., 2011) shows that the transition from inactive to active involves significant rearrangement of the helices, with a rotation about the main axis of transmembrane helix (TM) 6 producing displacement of the helix at the cytoplasmic end in the range of 10 Å when compared to the inactive starting structure. They hypothesise that the retinal induced movement of TM6 releases W6.48 (Ballesteros–Weinstein numbering convention, see Methods), which was previously interacting with D2.50, N7.49 and S7.45 via a cluster of water molecules, breaks a hydrophobic barrier which then enables the waters present here to form a hydrogen bonding network through the receptor to the two tyrosine residues Y5.58 and Y7.53 which have moved into the cytoplasmic cavity created by the rotation of TM6.

In contrast the group that crystallised the active state of β_2-AR (Rasmussen et al., 2011a) propose that activation is initiated by the ligand binding causing a 2 Å inward movement of TM5 around S5.46. They further hypothesize this inward movement of TM5 at the Pro bulge, P5.50, disrupts the network of interactions that exist between P5.50, I3.40, F6.44 and N7.45 which stabilises the receptor in an inactive state. This disruption of intra-molecular interactions results in a rotation of TM6 around F6.44 and the consequential outward movement of the cytoplasmic end of TM6.

Whilst both methods of activation appear feasible within the context of the systems being evaluated, neither provides a probable common activation pathway or a consensus framework which can then be further applied to all GPCRs in their activation by G proteins. However when these mechanisms are examined in greater detail together and analysed with all the active and inactive structures, including the muscarinic M_2 (M_2) (Haga et al., 2012; Kruse et al., 2013) and adenosine A_{2A} receptor (A_{2A}) (Jaakola et al., 2008; Dore et al., 2011; Lebon et al., 2011b; Xu et al., 2011) structures in the active and inactive conformations, a common mechanism is identifiable. This common activation mechanism, that draws upon and expands previously proposed mechanisms (Sheikh et al., 1996; Sheikh et al., 1999; Hulme et al., 2003), is generally applicable to all Family A GPCRs (Fig. 1). It is also consistent with the wealth of constitutively active mutant data and in-depth conservational analysis of significant residues implicated in the process. In its simplest form it involves the contact and movements of TM3 and TM6 relative to one another, however now with multiple active and inactive pairs of GPCRs available we are able to better understand at a molecular level what was originally proposed.

2. GPCR activation: a common mechanism

It is perhaps unsurprising that TM3 and TM6 are at the heart of any common activation pathway as they are in direct contact with every other helix except TM1 (Venkatakrishnan et al., 2013). Thus GPCR activation, whether initiated by ligands binding to the extracellular loops or to the primordial major and minor binding pockets (Rosenkilde et al., 2010), must in some way affect helices 3 & 6 to impart the common movements (Dore et al., 2011) seen within the active and inactive pairs available to date. Using the superposition of active and inactive states used in this study (defined in Methods) these common movements, shown in Fig. 1, are the movement of TM5, the slight rotation and upward movement of TM3, the rotation of TM6 and the inward movements of TM7 and TM1.

These movements of TM3 and TM6 may be facilitated, to some degree, by the breaking of the ionic interaction between the highly conserved residue R3.50 and an acidic residue in TM6 where this is present (Ballesteros et al., 2001). We argue, however, that a more important rearrangement is that of hydrophobic residues between TM3 and TM6 right in the core of the receptor (Fig. 2A). We have termed this central hydrophobic core of Family A GPCRs the "hydrophobic hindering mechanism" (HHM) because in the inactive state it serves to hinder the channel of water seen in active state crystal structures that facilitates the formation of the activated state. The HHM consists primarily of L3.43, F6.44, X6.40 (where X is a bulky hydrophobic residue, I, L, V or M) and to a lesser extent X6.41, and is arranged in the inactive state so that the L3.43 is held in place on top by F6.44 and to the side by X6.40, (Fig. 2A). The rearrangement of these contacts seen in all active state structures allows for the upward movement of TM3 along its axis and the rotation of TM6.

Following on from these movements of TM3 and TM6 the axial upward movement of TM3, and hence L3.43, is partially stabilised by the optimal hydrophobic packing with the highly conserved L2.46, (Fig. 2C). This optimal packing causes L2.46 to occupy the space of

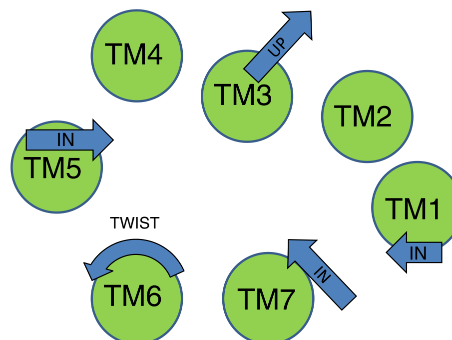

Fig. 1. Basic overview of the conformational changes seen in GPCR activation.

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