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Review

Yellow submarine of the Wnt/Frizzled signaling: Submerging from the G protein harbor to the targets☆

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ABSTRACT

The Wnt/Frizzled signaling pathway plays multiple functions in animal development and, when deregulated, in human disease. The G-protein coupled receptor (GPCR) Frizzled and its cognate heterotrimeric Gi/o proteins initiate the intracellular signaling cascades resulting in cell fate determination and polarization. In this review, we summarize the knowledge on the ligand recognition, biochemistry, modifications and interacting partners of the Frizzled proteins viewed as GPCRs. We also discuss the effectors of the heterotrimeric Go protein in Frizzled signaling. One group of these effectors is represented by small GTPases of the Rab family, which amplify the initial Wnt/Frizzled signal. Another effector is the negative regulator of Wnt signaling Axin, which becomes deactivated in response to Go action. The discovery of the GPCR properties of Frizzled receptors not only provides mechanistic understanding to their signaling pathways, but also paves new avenues for the drug discovery efforts.

Contents

1. Introduction: the sea of the Wnt/Frizzled signaling

Wnt signaling plays instructive roles in animal development, conserved from sponges to human beings, to activate the β-catenin-dependent transcriptional regulation of cell fate specification [1]. In the adult, this pathway is mostly silent. However, both improper overactivation and underactivation of this pathway can lead to diseases. Insufficient Wnt pathway activity underlies defects in tissue regeneration and the decreased proliferative potential of various stem cells [2,3]; it may also lead to certain neurodegenerative disorders [4]. On the other hand, misactivation of this signaling, e.g. through overproduction of the Wnt ligands or mutational activation of the downstream components of the pathway, promotes carcinogenesis, especially in the colon and breast [5,6]. In addition to this "canonical" β-catenin-dependent pathway, Wnt signaling also controls establishment of epithelial planar cell polarity (PCP). PCP is characterized by uniform polarization of the epithelial tissue within the plane of the epithelium, perpendicular to the typical apico-basal polarization of epithelial cells [7].

Frizzled (Fz) proteins have been identified as the receptors for the Wnt lipoglycoprotein ligands [8]. The first Fz cloned was that of

☆ The "Yellow Submarine" theme refers to the song of the same name from *The Beatles*.

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Drosophila, and the lab of Paul Adler disclosed the primary structure of this protein [9,10] as a 7-transmembrane helix (7-TM) receptor. This discovery happened 15 years after the pioneering works describing the existence of this transmembrane topology [11] and a decade since the prediction methods for this specific arrangement had emerged [12,13]. Reliability and simplicity of such methods based on the hydropathicity profile over the protein length allowed the 7-TM topology of the Fz protein to be no matter for any controversy. In contrast, the idea that 7-TM receptors may have the general feature of coupling to heterotrimeric G-proteins had been formulated only a few years earlier [14], but soon became widely accepted, and the words "7-TM receptors" and "G protein-coupled receptors (GPCR)" became essentially synonymous.

Heterotrimeric G proteins represent the immediate cytoplasmic transducers of GPCRs [15–17]. Upon binding of the agonist the receptor undergoes conformational changes, which enhance its GEF (guanine nucleotide exchange factor) activity towards the α-subunit of the heterotrimeric G protein. As a result, the α-subunit exchanges its GDP for GTP and dissociates from the βγ-heterodimer. Gα–GTP is then capable of interacting with downstream effectors until its intrinsic GTPase activity converts GTP back into GDP, leading to reassociation of the heterotrimeric complex, which brings the system to the "point zero". GPCRs interact with one or several types of G proteins, the specificity being determined by their α-subunits. Unlike more promiscuous βγ dimers [18], α-subunits are capable of signaling to specific effectors; the human genome contains 16 genes for different α-subunits constituting four families (Gαo/i, Gαq, Gαs and Gα12) [16]. Typically, any given cell expresses multiple types of heterotrimeric G-proteins [19].

Despite the growing body of evidence of GPCR coupling to heterotrimeric G-proteins, the data for the involvement of such transducers in Wnt/Fz signaling was missing for many years. Thus the G proteins were omitted from the emerging pathway architecture, being overshadowed by other major components of the cascade [8,20–22]. The first evidence which returned the missing G protein link in the chain of the Fz signal transduction was the role of pertussis toxin-sensitive G proteins obtained for the Fz-dependent non-canonical Ca^{2+}-pathway in zebrafish [23]. Later the Go-protein was found to be a transducer in both canonical and PCP pathways in Drosophila [24–26], and roles for its orthologues along with other members of the heterotrimeric GTPase superfamily in Xenopus [27,28] and mammalian models [28–31] of β-catenin stabilization as well as cell polarization [32] were established. Although these findings suggested that Fz proteins were activators of G-proteins, only recently the final biochemical proof of the ability of Fz receptors to bind heterotrimeric G-proteins and activate the nucleotide exchange on them upon engaging the Wnt ligands has been provided [33–35] – the final demonstration that these receptors are *bona fide* GPCRs [36–38]. Thus when all the i's are dotted and the t's are crossed, it is time to use the vast experience obtained in the field of GPCR functioning and pharmacology to bring us new concepts and insights into the work of the Fz pathway and ultimately to facilitate development of the drugs targeting it. This will be the focus of our review, followed by discussion of which effectors G proteins talk to upon transduction of the Wnt/Fz pathway.

2. Frizzleds as GPCRs: view from above the surface

GPCRs bear certain motifs in the extracellular and intracellular sequences, recognizable for their interactions with ligands and effectors. Fz receptors are no exception to this rule [39], and we proceed with description of the current knowledge of their features. On the extracellular side, Fz proteins carry a well-defined N-terminal cysteine-rich domain (CRD). The CRD contains ten cysteines conserved among the Fz receptor family [40]. This domain has been proposed as the main ligand-binding region of the receptor, and indeed a physical interaction of purified CRD with Wnt ligands has been demonstrated [41,42]. Additional proof for the role of CRD in Wnt binding comes from the fact that the so-called secreted Fz-related proteins (SFRPs), consisting essentially of just CRDs, serve as natural antagonists of Wnt signaling by competing with Fz for the interaction with Wnt ligands [43]. However, CRD is also present in the Smoothened receptor [44], which functions as a constitutive GPCR in the Hedgehog signaling pathway and has no influence on Wnt signaling. Additionally, experiments in Drosophila have demonstrated that a CRD-less Fz can efficiently rescue Fz loss-of-function alleles [45], suggesting that CRD is not the sole Wnt-binding region of the receptors. It is conceivable that a two-step ligand binding mechanism, known for some other GPCRs [46], also applies to Fzs, so that the Wnt-binding CRD serves to increase the local concentration of the ligand in the receptor's vicinity, while binding to the second site is required to trigger structural rearrangements in the GPCR and activate signal transduction.

Given the likely existence of several Wnt interaction regions in Fz receptors, the available information on the affinity of various Fz' CRDs for Wnt ligands [42] may not have a straightforward relevance for the physiological interactions between Wnts (19 in humans, 7 in Drosophila) and Fzs (ten in humans, four in flies). A systematic functional analysis of the Wnt–Fz interaction pairs is missing and requires an easy read-out system, measuring the most immediate events following the ligand–receptor interaction, rather than the final transcriptional response which is often affected by complicated feed-back regulations and depends on the exact composition of downstream elements of the cellular signaling machinery [47,48]. Until recently such read-outs were unavailable. Our experiments biochemically probing the GPCR activity of Fzs close this gap [33,34,49,50]. Indeed, the ability of a GPCR to catalyze guanine nucleotide substitution on the Gα-subunit is the most immediate consequence of the conformational change induced by ligand recognition, and can be easily measured. Using this assay, we have begun the systematic analysis of the Wnt–Fz coupling [33,34] and identified that human Fz1 is efficiently activated by Wnt3a and Wnt5a, Fz6 – by Wnt7a, Fz7 – by Wnt5a, and Fz10 – by Wnt3a. This set-up is also primed to be used in a high-throughput screening of small molecule antagonists of the Wnt–Fz interactions [50], the long-desired endeavor which might yield novel anti-cancer lead compounds [6].

Which factors apart from the intrinsic properties of the Fz GPCR may influence the specificity of the ligand binding? The mode of ligand interaction with a GPCR can be influenced by co-receptors – such as LRP5/6 for the canonical Fz signaling and Ror1/2 for the PCP signaling [51]. LRP5/6 are single-pass transmembrane proteins participating in a ternary complex with Wnt and Fz [52]. Recent evidence suggests that LRP5/6 may act as general GPCR accessory proteins, enabling non-Fz GPCRs to signal through the β-catenin pathway [53,54]. In this regard, LRP5/6 and Ror1/2 may be viewed as RAMPs (receptor-activity-modifying proteins): single-transmembrane accessory proteins regulating GPCRs [55].

Another potential level of complexity brought by LRP5/6 is their expected ability, as members of the lipoprotein receptor proteins, to interact with lipoprotein particles [56]. This becomes highly relevant in the context of Wnt signaling, as multiple forms of packaging of natural Wnt ligands have been proposed, including incorporation of the ligands into lipoprotein particles [57–59]. Wnts, being lipid-modified glycoproteins, easily bind to the outer cell membrane and extracellular matrix and are thus poorly diffusive as monomers [60], yet can migrate over long distances in vivo. It has been proposed that different ways to package Wnts serve to activate different groups of cells in the natural