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PERSPECTIVE

More Hints on Wnts: Gene Profiling by β_2-Adrenergic Receptor-Frizzled Chimeras

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The *Wnt-1* gene, the first member of a family of at least 18 different genes in mammals, was initially identified as the *Int1* oncogene. Based on its homology with the *Drosophila melanogaster wingless* gene, the term 'Wnt' was introduced (Nusse et al., 1991). Subsequently, it became clear that Wnt proteins (Wnts), secreted cysteine-rich glycoproteins of ~350 to 380 amino acids, are involved in the orchestration of key processes of animal development, including proliferation of stem cells, cell fate decisions, generation of cell polarity, development of the neural crest, and embryonic patterning. In the mature organism, Wnt signaling controls self-renewal of hematopoietic stem cells (Reya et al., 2003) and is implicated in human carcinogenesis (Miller et al., 1999). In 1996, members of the *frizzled* gene family were identified as putative Wnt receptors (Bhanot et al., 1996; Yang-Snyder et al., 1996); thus far, at least 10 different Frizzled receptors have been identified in the human genome (Fredriksson et al., 2003). The predicted *frizzled* gene products possess seven membrane-spanning domains, a key feature of G protein-coupled receptors, and a ~200-amino acid *N*-terminus with conserved cysteines implicated in Wnt binding. One of the most intriguing aspects of the Wnt-Frizzled system is its ability to govern highly diverse cellular processes, ranging from cell fate decisions and control of proliferation to cytoskeletal rearrangements, cell adhesion, and morphogenesis. Different Wnt-Frizzled combinations are likely to contribute to this diversity. Furthermore, one Frizzled receptor (e.g., Frizzled-7) can activate entirely different signaling pathways upon activation by different Wnt proteins (e.g., Wnt-8b and Wnt-11).

Three major Wnt-Frizzled signaling pathways have been identified (Fig. 1A). In the so-called canonical pathway, the regulation of the fate and function of β-catenin is at the center of Wnt-Frizzled signaling. In the inactive state, β-catenin is phosphorylated by glycogen synthase kinase-3 and inactivated by ubiquitination and proteasome-mediated degradation. Activation of Frizzled receptors by Wnts inactivates glycogen synthase kinase-3 via the phosphoprotein called "dishevelled", resulting in stabilization of β-catenin, which then escapes degradation and translocates to the nucleus to interact with lymphoid-enhancer factor/T cell factor class proteins, members of the high-mobility group family of transcription factors (Cook et al., 1996; Larabell et al., 1997). The second Wnt-Frizzled signaling pathway, the planar polarity pathway, results in a β-catenin-independent activation of the c-Jun NH_2-terminal kinase cascade and involves dishevelled and small GTPases of the Cdc42/Rho family. The final effect of this pathway is the polarization of the cytoskeleton and the activation of the transcription factor Jun (Heisenberg et al., 2000; Tada and Smith, 2000). Finally, for Frizzled-2 (upon stimulation by Wnt-5a) and for Frizzled-7, a Ca^{2+} pathway has been described that apparently involves phospholipase C and phosphodiesterase isozymes. This pathway has been implicated in cell movement processes required for embryonic patterning (Slusarski et al., 1997; Sheldahl et al., 2000; Ahumada et al., 2002).

This scenario of Wnt-Frizzled signaling became more complex by the discovery of low-density lipoprotein receptor–related proteins 5 and 6 (LRP5 and LRP6) as coreceptors for certain Wnt proteins. Wnts bind to the extracellular domain of these proteins, which interact intracellularly with the scaffolding protein axin. Combined activation of LRP5 or LRP6 with Frizzled receptors seems to be important for activation of the canonical β-catenin cascade. Whether LRP5 and LRP6 are also involved in the Ca^{2+} or the planar polarity pathways remains to be established. Available evidence suggests that not all Frizzled receptors interact with LRPs and that such dimers do not bind all Wnts, which could generate increased specificity (Tamai et al., 2000; Wehrli et al., 2000). Additional modulators of the Wnt-Frizzled system have been identified that act as Wnt inhibitors. Among these are secreted Frizzled receptors and proteins such as Cerberus, Dickkopf, and WIF (Wnt inhibitory factor), which either bind specific Wnt proteins or block, as shown for Dickkopf-1, the interaction of Wnts with LRPs (Semenov et al., 2001). Taken together, a complex scenario of different agonists, receptors, coreceptors, and antagonists con-

ABBREVIATIONS: LRP, low-density lipoprotein receptor-related protein; β_2AR, β_2-adrenergic receptor.

tributes to the temporal and spatial regulation of Wnt-Frizzled signaling required for proper animal development.

Wnt-Frizzled research has been severely hampered by the absence of purified endogenous or recombinant Wnt proteins. Only very recently, Willert et al. (2003) succeeded in the first purification of biologically active Wnt proteins. They further showed for Wnt-3a and *D. melanogaster* Wnt-8 that Wnts carry an essential palmitoylation on a highly conserved cysteine residue. So far, the only way to stimulate Frizzled receptors has been to use conditioned media from cultured cells overexpressing defined Wnts. Such cell supernatants, however, are rather crude tools, because they require careful experimental controls, are difficult to standardize, and may be contaminated with other agonists potentially coproduced and secreted by the Wnt-overexpressing cells.

How to overcome this situation? The ingenious solution was 'Let Frizzleds react with catecholamines'! In this issue of *Molecular Pharmacology*, Li et al. (2004) present exciting data on gene expression profiles induced by catecholamine-responsive Frizzled-1 and Frizzled-2 receptors. Based on previous work on chimeras of classic G protein-coupled receptors (Kobilka et al., 1988; Wong et al., 1990), the authors generated β_2-adrenergic receptor (β_2AR)-Frizzled chimeras, consisting of the extracellular and transmembrane domains of the β_2AR and the putative intracellular loops of the rat Frizzled-1 (Rfz1) or Frizzled-2

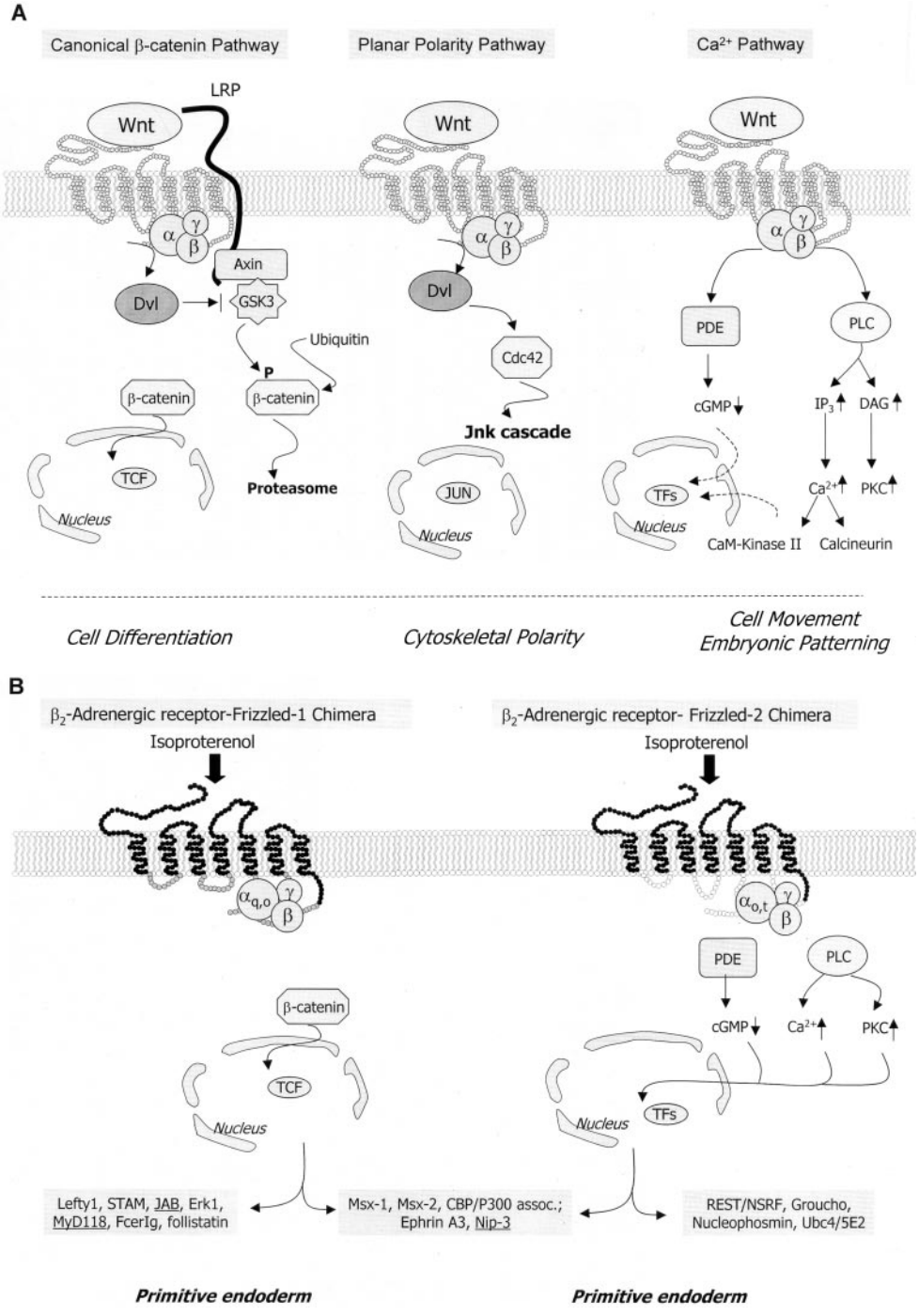

Fig. 1. A, major signaling pathways identified for certain Wnt-Frizzled combinations. B, gene expression by chimeras of the β_2-adrenergic receptor (black) and Frizzled-1 (gray) or Frizzled-2 (white) in F9 teratocarcinoma cells, as reported by Li et al. (2004). The genes up-regulated by these chimeras in response to isoproterenol are given in the gray boxes; those involved in primitive endoderm formation are underlined. Dvl, dishevelled; GSK3, glycogen synthase kinase-3; LRP, low-density lipoprotein receptor-related protein; TCF, lymphoid-enhancer factor/T cell factor class transcription factor; PDE, phosphodiesterase; PLC, phospholipase C, PKC, protein kinase C; IP_3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; TFs, transcription factors; CaM-Kinase II, Ca^{2+}/calmodulin-dependent protein kinase II.

receptor (Rfz2). In a series of recent articles from the same group (Liu et al., 1999, 2001; Ahumada et al., 2002; deCostanzo et al., 2002), it was demonstrated that these receptor chimeras (β_2AR/Rfz1 and β_2AR/Rfz2) bind typical β_2AR ligands but are unable to stimulate adenylyl cyclase. Activation by isoproterenol of the β_2AR/Rfz1 chimera expressed in mouse totipotent F9 teratocarcinoma cells resulted in primitive endoderm formation via the canonical β-catenin pathway. This action of the β_2AR/Rfz1 chimera, which did not induce the Ca^{2+} pathway, was apparently mediated by $G\alpha_o$ and $G\alpha_q$. Thus, catecholamine activation of the β_2AR/Rfz1 chimera mimicked the effects of Wnt-8 in Rfz1-expressing cells. On the other hand, catecholamine activation of the β_2AR/Rfz2 chimera did not lead to stabilization of β-catenin, but activated the Ca^{2+} signaling pathway, as reported for Rfz-1 activated by Wnt-5 (Fig. 1B).

In the present article, the authors use these β_2AR/Rfz chimeras to analyze the specific genes activated by Frizzled-1 and Frizzled-2. A major merit of this study is that the gene expression profile was monitored over a long (45 h) period after stimulation with β_2AR agonist and that most results were confirmed by quantitative reverse transcription-polymerase chain reaction and protein expression analysis. Furthermore, for some genes the biological plausibility of the findings was validated by antisense experiments, showing that knocking-out up-regulated genes inhibited primitive endoderm formation stimulated by the β_2AR/Rfz1 chimera. As with all gene profiling studies, the authors were overwhelmed with vast amounts of data. To overcome this problem, they chose to consider only genes that were up-regulated by 1.5-fold. Setting such a threshold creates the risk that one neglects relevant or essential genes up-regulated by less than 50% or that are suppressed, caveats that pertain not only to this but to most gene expression profiling studies. Nevertheless, this strategy identified 12 and 9 genes up-regulated by β_2AR/Rfz1 and β_2AR/Rfz2, respectively (Fig. 1B). From the known functions of these genes (transcription factors, proteins involved in signal transduction, embryonic patterning, and organogenesis), these results seem biologically plausible. Furthermore, several of the up-regulated genes identified here match results of a recent study by Willert et al. (2002). Differences in the expression profiles of the two studies may relate to the fact that those authors used Wnt-3a as stimulus and a cell line (NCCIT) expressing other Frizzled receptors.

Taken together, the studies with the β_2-adrenergic receptor-Frizzled chimeras have provided us with a wealth of exciting data on Wnt-Frizzled signaling, reaching from early signaling events to cell physiology and gene expression. With the advent of suitable purification methods for Wnt proteins, it will be now possible to confirm these data and to address issues for which these receptor chimeras are inherently unsuitable (e.g., the roles of LRP coreceptors for early Wnt signaling and the Frizzled receptor specificities for Wnt isoforms). Thus, although β_2- adrenergic receptor-Frizzled chimeras have provided a highly useful way to advance understanding of Wnt-Frizzled signaling, still more hints on Wnt's actions are needed to fully comprehend their roles in physiology and pathology.

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