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*J Immunol* 2008; 181:4441-4445; doi: 10.4049/jimmunol.181.7.4441

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Transcriptional Outcome of Wnt-Frizzled Signal Transduction in Inflammation: Evolving Concepts

Malini Sen*† and Gourisankar Ghosh†

Wnt-Frizzled signaling was first identified as a key event in Drosophila development. Over the years, ample evidence has accumulated regarding the multiple roles of Wnt-Frizzled signaling in mammalian cell differentiation and tissue/organ morphogenesis. It is thus not surprising that variations in the regulatory network of the Wnt signaling scheme would lead to alterations in cellular organization and cell activation and to the development of pathogenic conditions. Several reports have accordingly implied the involvement of Wnt-Frizzled signaling in the activation of proinflammatory mediators in inflammatory disorders. We will discuss how Wnt-Frizzled signaling may initiate/augment inflammation, focusing on its transcriptional outcome. The Journal of Immunology, 2008, 181: 4441–4445.

Signal transduction of Wnt-Frizzled is an important aspect of tissue/organ development and maintenance. Wnt (glycoprotein ligands) and Frizzled (Fz) receptor (transmembrane Wnt receptors) interact with other structural components at the cell surface to initiate complex signal transduction cascades that culminate in the transcriptional regulation of gene expression. The consequences are diverse, ranging from cell proliferation/differentiation to cell adhesion and migration and tissue/organ morphogenesis. Thus, aberrant Wnt signaling may promote disarray in cell/tissue organization, leading to various pathogenic outcomes.

Wnt and Fz family genes were first cloned and characterized in Drosophila. Mammals express 19 or more Wnt ligands and 12 or more Fz receptors. There being considerable homology among the Wnt ligands and Fz receptors, cross-reactivity between Wnt-Fz interactions is quite frequent (1–3). Wnts harbor potential sites for glycosylation and palmitoylation, which appear to be useful for signal transduction (1–5). The relative cellular distribution of Wnts in different cell types has not been deciphered. Although secreted, on account of posttranslational modifications it is possible that a considerable fraction of Wnt proteins remains membrane or extracellular matrix bound. Based on hydropathy plot analysis, the Fz receptor family comprises seven hydrophobic α-helices that span the lipid bilayers, three intracellular loops, three extracellular loops, an extracellular N-terminal segment that carries a cysteine rich domain, and an intracellular C-terminal tail. Topologically, the Fz receptors are similar to the heterotrimeric G protein-coupled receptors such as the β-adrenergic receptor (1, 3, 6, 7). Comparative bioinformatic analysis of the intracellular loops of >100 G protein-coupled receptors and the Fz receptors suggests that there is differential coupling of the Fz receptors with heterotrimeric G proteins, which constitute the Go, Gi, Ga, Gb, and Gd subtypes of Ga subunits (7, 8). Although Wnt-Fz interaction in mammals has not been biochemically demonstrated to be directly coupled to heterotrimeric protein activation, chimeric receptors harboring the intracellular loops of rat Fz1 or Fz2 and the transmembrane and extracellular regions of the β-adrenergic receptor have been shown to activate the Go, Gi, and Ga classes of heterotrimeric G proteins when stimulated by β-adrenergic receptor agonists (2, 6). Additionally, Fz receptors have a conserved “KTXXXW” motif near their C terminus that is known to bind to the conserved PDZ (Psi-95/Disc large and ZO-1) domain of the cytoplasmic protein Disheveled (Dvl) during intracellular signaling events initiated by Wnt-Fz interactions (7) (Fig. 1).

Wnt signal transduction pathways have been broadly categorized into two types, canonical and noncanonical. Contemporary literature provides sufficient evidence of both signaling pathways as being important in health and disease. Despite the discovery of several signaling intermediates of the Wnt-Fz signaling scheme, the detailed molecular mechanism of the transcriptional outcome of Wnt-Fz signaling remains unresolved. This review will discuss the current concepts of Wnt-Fz signaling with special emphasis on the noncanonical mode, focusing on its transcriptional outcome.

Current concepts of Wnt-Fz signaling

Canonical Wnt-Fz signaling has been studied more elaborately than noncanonical signaling. Concepts of the canonical Wnt-Fz
particular Wnt will emerge as canonical or noncanonical (mediate β-catenin-mediated transcription or not) depends to a large extent on the Fz receptor it encounters (10). With reference to studies on Wnt-Fz interaction performed in organisms ranging from Drosophila to mouse and human cell lines, putative Wnt-Fz ligand receptor pairs, summarized in Table I, have been identified.

Several Wnt-Fz interactions such as Wnt5a-Fz2, Wnt5a-Fz5, etc. of the noncanonical pathway are associated with increases in intracellular calcium levels that lead to the activation of calcium-sensitive enzymes such as PKC and CaMK (9, 13, 14). The precise mechanism of Ca2⁺ induction is unclear. Recent reports suggest the establishment of a link between the Fz receptor and phospholipase C (PLC) following Wnt-Fz interaction (15). It is possible that dissociation of the Fz receptor-coupled heterotrimeric G protein subsequent to Wnt-Fz binding triggers PLC activity, leading to augmented intracellular calcium stores and subsequent activation of PKC and CaMK. Although the molecular mechanism in different cell types is unclear, there is evidence that the noncanonical Wnt/calcium pathway antagonizes the canonical Wnt signaling pathway through the inhibition of β-catenin-mediated transcriptional activation (14). Several reports have suggested that noncanonical Wnt-Fz signaling operates also independently of alterations in intracellular calcium concentrations (10, 12). It is, however, not clear how transcriptional activation is attained in such scenarios. Ca2⁺-independent PKC isoforms and MAPKs such as Erk1/2 could operate therein. Erk1/2 activity in response to Wnt5a signaling has in fact been indicated in osteoblastic cells (16).

Cytoplasmic Dvl, which is differentially phosphorylated upon activation through Wnt-Fz interaction, may adopt multiple conformations that guide specific interactions with other cytoplasmic proteins. It has been suggested that during noncanonical Wnt signaling, the DEP (Disheveled/Egl-10/Pleckstrin) domain of activated Dvl interacts with Diversin and thereby transmits signals to the Rho family of GTPases (17). Whether Dvl DEP domain-mediated interactions are distinct or associated with Fz receptor-coupled heterotrimeric G protein activity remains unclear. Nevertheless, Rho activation may stimulate JNK activity (18) and perhaps also induce PKC translocation and activity via cytoskeletal alterations.

An additional layer of regulation of noncanonical Wnt signaling arises from the ability of Wnt5α-like ligands to interact with retinoic acid-related orphan receptor (ROR) family cell-surface tyrosine kinase receptors. The ROR family is characterized by the presence of the extracellular Fz-like cysteine-rich and kringle domains, which are known for their ability to bind Wnt ligands. Recent reports suggest that Wnt5α-ROR2 interaction leads to the activation of Jun kinase via Dvl (19, 20).

It is not yet established whether the lipoprotein receptor-related protein (1–3), which is a costimulator during Wnt-Fz interaction in the canonical Wnt pathway, is also required for noncanonical Wnt signaling pathway have already been detailed in excellent reviews (1, 2). In brief, in the canonical pathway, Wnt-Fz interaction with the low density lipoprotein receptor-related protein, LRP, at the cell surface initiates a signal transduction cascade that leads to the inactivation of the kinase glycogen synthase kinase-3β (GSK3β) and the concomitant stabilization of the transcriptional coactivator protein β-catenin in the cytosol. β-Catenin eventually translocates to the nucleus and forms the transcriptionally active β-catenin:lymphoid enhancing factor (LEF); T cell factor (TCF) complex on the promoters of target genes. Noncanonical Wnt signaling, in contrast, comprises a combination of signaling intermediates such as protein kinase C (PKC) and calmodulin kinase (CamK) that can operate independently of β-catenin activation after signaling is initiated by Wnt-Fz interaction (Fig. 1). Interestingly, Dvl, which gets phosphorylated upon Wnt-Fz interaction, is at the crossroads of noncanonical and canonical Wnt signaling pathways (7). However, the temporal and spatial association between G protein and Dvl activation during Wnt-Fz signaling is not very clear.

The course of Wnt-Fz signaling is dictated by the specificity of Wnt-Fz interactions, which is probably governed by both the cell type and the stage of cell growth or differentiation (1–3, 9–13). Wnt1, Wnt3a, Wnt2, and Wnt10B, which are known to promote β-catenin-dependent gene transcription, are termed the canonical Wnts. Other Wnts such as Wnt5a and Wnt11, which can mediate transcriptional activation that is β-catenin independent, are usually termed the noncanonical Wnts. Importantly, however, whether a

Table I. Putative Wnt-Fz ligand receptor pairsa

<table>
<thead>
<tr>
<th>Wnt</th>
<th>Wnt-Interacting Fz</th>
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<tbody>
<tr>
<td>Wnt2 (mouse)</td>
<td>Fz9 (rat)</td>
</tr>
<tr>
<td>Wnt1/Wingless (Drosophila)</td>
<td>Fz4 (mouse), Fz/Fz2 (Drosophila)</td>
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<tr>
<td>Wnt4 (mouse)</td>
<td>Fz3 (mouse)</td>
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<tr>
<td>Wnt5α (human, mouse, Xenopus)</td>
<td>Fz5 (human, mouse), Fz2 (rat), Fz4 (mouse)</td>
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<tr>
<td>Wnt8 (Xenopus)</td>
<td>Fz7 (mouse), Fz8 (mouse), Fz1 (rat)</td>
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a This table is based on information provided in Refs. 1, 2, 10, 11, 15, 22, and 25 and on the Wnt website (www.stanford.edu/~muse/wif/ffz/wwnt.html).
signaling. Also uncertain is whether the secreted Fz-related proteins (sFRP/DKK), which are known to antagonize canonical Wnt signaling, regulate the noncanonical pathway as well (1–3, 21).

Although Wnt signaling has been divided into the canonical and noncanonical types, a crosstalk between the noncanonical and the canonical signaling intermediates is not unusual and is in fact of frequent occurrence in several cellular contexts (1–3, 10, 22). Perhaps the Wnt receptors of a particular cell type, which couple to intracellular mediators of signal transduction in their active conformations, play a major role in signal specificity determination.

Noncanonical Wnt signaling; transcriptional outcome, and inflammation

Several intermediates of noncanonical Wnt signal transduction have been identified in different cell types. However, the differential activation of the signaling intermediates in response to Wnt-Fz interactions and the respective transcriptional outcomes have not yet been clearly dissected. Since the first report of up-regulated expression of cytokines such as interleukins 6, 8, and 15 by Wnt5a in rheumatoid arthritis (RA) synovial fibroblasts (23), there has been a surge of interest in Wnt signaling in the context of inflammation in pathogenic disorders (24, 25). Recently, it has been reported that Wnt5a-Fz5 signaling promotes IL-12 synthesis and enhances the inflammation induced by microbial stimulation in macrophages (26). Another report further shows that the noncanonical Wnt5A-Fz5 pathway acts through CaMKII to sustain inflammation (25). The same report demonstrated that human macrophages treated with LPS and TNF-α markedly activate the expression of Wnt5a. Similar signaling schemes have been implicated in the progression of different types of cancers such as melanoma, where inflammation appears to be a crucial component in the disease course (27, 28). Interestingly, increased Wnt5a expression has recently been demonstrated in psoriasis, another inflammatory disorder characterized by enhanced cytokine synthesis (29). It remains to be investigated however, whether there is a direct cause and effect connection between Wnt5a signaling and the transcriptional activation of proinflammatory cytokine synthesis in melanoma and psoriasis. Current concepts regarding the overall scheme of Wnt signaling have been summarized in Fig. 1.

In this context, it is worthwhile to mention that LEP-1/β-catenin has been found to promote the transcription of proinflammatory mediators such as COX2 and MMP13 (30, 31). It remains to be seen, however, whether before LEP-1/β-catenin-mediated transcription there is any crosstalk between intermediates of the canonical and noncanonical Wnt signaling pathways. The following sections highlight the modes of noncanonical Wnt signaling that potentially activate the NF-κB, NFAT, and API family transcription factors.

NF-κB. The NF-κB family consists of five members: p50, p52, p65 (also known as RelA), c-Rel, and RelB, which form combinatorial dimers. The NF-κB family transcription factors constitute one of the most common mediators of transcription leading to chemokine/cytokine synthesis concomitant with chronic inflammatory disorders such as RA (32, 33). The rapid and transient activation of the RelA and c-Rel dimers during inflammation is contrasted by the slow and sustained activation of RelB dimers (34). The activity of the RelB:p52 heterodimer may be important for cell survival/differentiation during secondary lymphoid tissue-like organization in the setting of sustained inflammation and adaptive immunity (35). Several reports have accordingly suggested that RelB is important for the differentiation of APCs such as dendritic cells (36). Recent studies implicate the two different NF-κB activation schemes (either rapid or slow/sustained) as being intertwined and may rely on each other for promoting chronic inflammation (37, 38) (Fig. 2).

In light of the suggested link between Wnt5a-Fz5 signaling and induction of the NF-κB responsive inflammatory cytokines IL-6, IL-8, and IL-15 in the synovial fibroblasts of RA and psoriasis patients (11, 23), it is plausible to propose that Wnt5a-Fz5 signaling promotes inflammation in RA at least partly through the activation of NF-κB-mediated gene transcription. In fact, our experimental data on NF-κB reporter gene induction in synovial fibroblasts has demonstrated that increased expression of Wnt5a or Fz5 enhances NF-κB activity (M. Sen, unpublished results). Given the considerable homology among several Wnt and Fz homologues, it is also possible that Wnt5a-like ligands interact with Fz isoforms other than Fz5 on the cell surface and initiate signaling cascades leading to NF-κB activation and inflammatory gene induction.

A recent report has demonstrated that ROR1 is expressed at high levels in chronic lymphocytic leukemia cells but not in nonleukemic cells and that enforced expression of ROR1 in 293 T cells induces NF-κB via direct binding to Wnt5a (39). It is likely that aberrant ROR1 expression provides a chronic survival signal in chronic lymphocytic leukemia cancer cells through a Wnt5a-ROR1-NF-κB pathway.

Given that noncanonical Wnt signaling is associated with PKC/CaMKII activation and that both PKC and CaMKII can promote NF-κB-mediated transcription (9, 13, 40), it is possible that noncanonical Wnt signaling contributes to the synthesis of NF-κB-responsive inflammatory mediators via PKC and/or CaMKII activation.
**NFAT.** Another important transcriptional regulation in noncanonical Wnt signaling appears to be through the modulation of NFAT activity. In unstimulated cells, NFAT is phosphorylated by GSK3β and remains in the cytoplasm as an inactive phosphoprotein where the phosphorylated acidic segment masks the basic nuclear localization sequence. Ca2⁺-mediated activation of the protein phosphatase calcineurin dephosphorylates NFAT and induces its nuclear translocation (41). Wnt5a-Fz-Ca2⁺ signaling may accordingly orchestrate a gene expression program through NFAT by an increase in intracellular Ca2⁺ concentration. In fact, in T lymphocytes, Wnt5a-Fz5 interaction has been suggested to promote nuclear localization of unphosphorylated activated NFAT through the inactivation of GSK3β (22). A parallel theme as a consequence of GSK3β inactivation could be the nuclear translocation and activation of β-catenin. In such a scenario, how a balance would be maintained between the use of GSK3β for β-catenin-dependent and -independent effects is currently undocumented. Wnt5a signaling has also been implicated in suppressing NFAT activation through the up-regulation of Yes-Cdc42-casein kinase activity (42). Although not clearly demonstrated, perhaps PKC activation as a consequence of Wnt5a signaling also partakes in synergy with other pathway(s) to regulate NFAT activity. An unregulated increase in NFAT activity could lead to heightened production of NFAT-responsive cytokines such as IL-2 and promote inflammation. In fact, several lines of evidence implicate the synergism of NFAT family members with transcription factors of the NF-κB and AP1 families in different inflammatory disorders (43, 44).

**AP1.** Noncanonical Wnt signaling through Wnt5a-ROR interactions as mentioned earlier may result in the up-regulation of AP1 (Fos/Jun) activity through the activation of MAPKs such as JNK and Erk1/2. JNK and Erk1/2 activity may be induced by the Wnt5a-Fz pathway through the activation of Dvl and several G proteins including Rac and Rho (45, 46). Perhaps AP1 activity also arises in conjunction with the inhibition of canonical Wnt signaling through Wnt5a/Ca2⁺-mediated activation of TAK (TGFβ-activated kinase 1), MAP3K (MAPK kinase kinase), and NLK (Nemo-like kinase), a mitogen-activated protein kinase (14). Several proinflammatory cytokine and survival genes contain AP1 binding sites along with other transcription factor binding sites in their promoters. Cooperative transcription by NF-κB and AP1 may thus be possible in certain instances, as reported in the transcriptional activation of the proinflammatory cytokines IL-6 and IL-8 (47–49). It is however unclear whether these two classes of transcription factors promote cytokine gene expression by binding to their respective promoters independently or in an interdependent mode.

**Regulation of noncanonical Wnt signaling**

Although Wnt5a signaling has been implicated in inflammation as in RA, psoriasis, and inflammatory cancers, it is not clearly understood what initiates or promotes the signaling pathway during disease pathogenesis. Recent observations regarding the activation of noncanonical Wnt5a signaling in response to microbial stimulation is perhaps an important clue in this respect (26). To address the question of transcriptional regulation of Wnt-Fz, it is important to identify potential transcription factor binding sites within the promoter and enhancer elements of the target Wnt-Fz genes and evaluate the relative kinetic profiles of the binding of these transcription factors to the respective promoter/enhancer sites. Wnt5a promoter analysis has revealed several SP1, AP1, and AP2 binding sites and several CpG motifs (50). Wnt5a expression patterns in different cell types could thereby be controlled by the extent of AP1/AP2 activity and promoter methylation/demethylation. Wnt5a signaling could accordingly be initiated or augmented by oxidative stress, which can potentially promote AP1/AP2 activity and promoter demethylation via installation of the appropriate signaling intermediates. In addition to the identification of AP1/AP2 sites in Wnt promoters, binding sites for the helix-loop-helix transcription factor POU domain protein have been identified in Fz homologues (51). It is however unclear as to how the entire transcriptional program for Fz gene expression is orchestrated.

The report by Blumenthal et al. is first to show the possible involvement of the NF-κB transcription factor in the induction of Wnt5a expression through the TLR pathway (26). Consistent with this model, the *Drosophila* WntD protein, which is involved in the maintenance of innate immune responses, is activated by the Toll/Dorsal (Dorsal is the NF-κB homologue in fly) signaling pathway (52). However, no NF-κB binding site has been reported in the promoters of either Wnt or Fz genes. Therefore, it is unclear whether NF-κB is involved in the transcription of mammalian Wnt and Fz homologues as a primary or a secondary mediator. The several reports cited here however suggest an intriguing possibility where a Wnt-Fz-NF-κB regulatory loop might be responsible for maintaining high levels of inflammatory cytokines in inflammatory diseases (Fig. 2).

In the context of regulation of Wnt gene expression, reference may also be made of a recently identified HOX transcriptional program that mediates the expression of Wnt5a (53). Sufficient information is not available to evaluate the prominence of HOX transcription factors during the initiation or progression of inflammation. Nevertheless, existing data suggest that alterations in HOX gene expression profiles may promote predisposition to immunological abnormalities.

**Conclusions**

With the emerging importance of noncanonical Wnts such as Wnt5a in inflammatory disorders, the regulation and molecular mechanism of noncanonical signaling during pathogenesis have begun unfolding. Although several signaling intermediates are being implicated in the propagation of signals generated at the cell surface in different cell types, it is not clear how these intermediates crosstalk or precisely how the transcriptional outcome is programmed. Some outstanding questions persist: 1) how are the expression profiles of Wnt and Fz proteins regulated; 2) what decides whether GSK3β inactivation will lead to noncanonical or canonical signaling; 3) what is the relative importance of the transcription factors NFκB, NFAT, and AP1/AP2, all which have been implied in noncanonical Wnt signaling; and 4) what are the precise mechanisms of activation of these transcription factors and how is their specificity determined? In this context, it is of interest to clearly investigate how NF-κB is induced during the immune modulations associated with chronic inflammation. For instance, the differentiation of Ag-presenting dendritic cells or the maturation of B cells to Ab-secreting plasma cells (11) in chronic RA, may be attributed, at least partly, to distinct NF-κB isoforms. A better resolution of the transcriptional outcome will lead to an improved understanding of the relevance of Wnt5a and other Wnts partaking of noncanonical signaling in the pathogenesis of inflammatory disorders.

**Acknowledgments**

We thank Dustyn Miller for the figures.
Disclosures

The authors have no financial conflict of interest.

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