WNT factors represent key mediators of many processes in animal development and homeostasis and act through a receptor complex comprised of members of the Frizzled and low density lipoprotein-related receptors (LRP). In mammals, 19 genes encoding Wingless and Int-related factor (WNTs), 10 encoding Frizzled, and 2 encoding LRP proteins have been identified, but little is known of the identities of individual Frizzled-LRP combinations mediating the effects of specific WNT factors. Additionally, several secreted modulators of WNT signaling have been identified, including at least three members of the Dickkopf family. WNT7A is a WNT family member expressed in the vertebrate central nervous system capable of modulating aspects of neural plasticity. Gene knockout models in the mouse have revealed that WNT7A plays a role in cerebellar maturation, although its function in the development of distal limb structures and in the reproductive tract have been more intensely studied. To identify a receptor complex for this WNT family member, we have analyzed the response of the rat pheochromocytoma cell line PC12 to WNT7A. We find that PC12 cells are capable of responding to WNT7A as measured by increased β-catenin stability and activation of a T-cell factor-based luciferase reporter construct and that these cells express three members of the Frizzled family (Frizzled-2, -5, and -7) and LRP6. Our functional analysis indicates that WNT7A can specifically act via a Frizzled-5-LRP6 receptor complex in PC12 cells and that this activity can be antagonized by Dickkopf-1 and Dickkopf-3.

Members of the WNT gene family encode structurally related secreted glycoprotein factors, modulating a vast array of processes during vertebrate and invertebrate embryonic development as well as several aspects of tissue homeostasis in the adult (1–4). In embryos, signaling by WNT factors controls the organization of the body plan during the early stages of development as well as organogenesis at later developmental stages. Postnatally, WNT signaling is involved in normal biological processes in animal development and homeostasis and act through a receptor complex comprised of members of the Frizzled and low density lipoprotein-related receptors (LRP). In mammals, 19 genes encoding Wingless and Int-related factor (WNTs), 10 encoding Frizzled, and 2 encoding LRP proteins have been identified, but little is known of the identities of individual Frizzled-LRP combinations mediating the effects of specific WNT factors. Additionally, several secreted modulators of WNT signaling have been identified, including at least three members of the Dickkopf family. WNT7A is a WNT family member expressed in the vertebrate central nervous system capable of modulating aspects of neural plasticity. Gene knockout models in the mouse have revealed that WNT7A plays a role in cerebellar maturation, although its function in the development of distal limb structures and in the reproductive tract have been more intensely studied. To identify a receptor complex for this WNT family member, we have analyzed the response of the rat pheochromocytoma cell line PC12 to WNT7A. We find that PC12 cells are capable of responding to WNT7A as measured by increased β-catenin stability and activation of a T-cell factor-based luciferase reporter construct and that these cells express three members of the Frizzled family (Frizzled-2, -5, and -7) and LRP6. Our functional analysis indicates that WNT7A can specifically act via a Frizzled-5-LRP6 receptor complex in PC12 cells and that this activity can be antagonized by Dickkopf-1 and Dickkopf-3.

Members of the WNT gene family encode structurally related secreted glycoprotein factors, modulating a vast array of processes during vertebrate and invertebrate embryonic development as well as several aspects of tissue homeostasis in the adult (1–4). In embryos, signaling by WNT factors controls the organization of the body plan during the early stages of development as well as organogenesis at later developmental stages. Postnatally, WNT signaling is involved in normal biological processes such as tissue maturation and homeostasis and in several neoplastic pathologies (2, 5–8). For example, in the mammalian central nervous system (CNS)1 WNT signal transduction is involved in neural induction and patterning in early embryogenesis (2, 4) as well as in organogenesis and neuronal homeostasis at later stages (9). In the adult, WNTs play a role in the control of neuronal plasticity and are implicated in CNS neoplasias such as medulloblastoma (10–14). The analysis of the signaling events mediated by WNTs has uncovered at least three signal transduction pathways, each involved in the mediation of specific biological responses (2, 15). The most studied and best understood signaling cascade elicited by WNTs involves an interaction with a receptor complex comprising members of the Frizzled (FZD) class of 7-transmembrane receptors and a member of the low density lipoprotein receptor (LRP) family of single-pass membrane proteins (16). WNT interaction with its receptor results in an increase in the stability of β-catenin, whose accumulation results in translocation to the nucleus where it can interact with members of the TCF class of transcription factors and therefore modulate gene expression. The stability of β-catenin is controlled by WNT through the modulation of a large cytoplasmic protein complex comprised of the proteins AXIN, APC, GBP/FRAT, and GSK3β, the latter controlling directly the level of β-catenin phosphorylation and its consequent degradation by the proteasome pathway (2, 17). WNT action can also be modulated at the extracellular level by several classes of secreted factors, including members of the Dickkopf, Cerberus, and FRP protein families (2, 18). The vast array of processes controlled by WNTs is reflected in the numerous mammalian WNT and Frizzled classes, numbering 19 and 10 members, respectively, in man (2). During development, many of these genes are expressed in a temporally and spatially regulated fashion, often in overlapping domains, whereas others are more widely expressed (19–21). These observations suggest a degree of specificity of action of the individual ligands and of relative affinities in the ligand-receptor interactions. Given the wider expression patterns of LRP genes and the tissue-specificity of expression displayed by many of the more numerous FZD genes, the specificity of the cellular response to individual WNT ligands is likely to depend largely on the individual FZD proteins expressed on the cell membrane. Despite the fact that a number of studies have investigated the association between individual Wnts and Frizzled proteins (22–28) and of the wealth of information on the biological responses elicited by WNTs in different models systems, rela-

1 The abbreviations used are: CNS, central nervous system; LRP, low density lipoprotein; ERK, extracellular signal-regulated kinase; Fzd, Frizzled; Wnt, Wingless and Int-related; Tcf, T-cell factor; Frp, Frizzled-related protein; Dkk, Dickkopf.
tions or in the presence of 10 mM LiCl for 24 h) were clarified by centrifugation (10,000 g for 15 min. The cell lysates (PC12 cells cultured under standard conditions) were lysed in Triton X lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM sodium orthovanadate, 50 mM sodium fluoride, and 10 mM β-glycerophosphate) for 15 min. The cell lysates (PC12 cells cultured under standard conditions or in the presence of 10 mM LiCl for 24 h) were clarified by centrifugation (10,000 × g for 10 min). Protein cell lysates (80 μg) were separated by SDS-polyacrylamide gel electrophoresis, blotted onto nitrocellulose membranes, and probed using polyclonal anti-β-catenin antibody (sc-1496; Santa Cruz Biotechnology) at 1 μg/ml dilution. Membranes were also probed with polyclonal anti-ERK antibody (sc-93; Santa Cruz Biotechnology) at 0.5 μg/ml to control equal protein loading.

**Key Figures:**
- **Fig. 1.** The canonical WNT signaling pathway is activated in PC12 cells in response to WNT7A. Western blotting analysis of total cellular protein from PC12 cells transfected with empty vector or with an expression plasmid encoding WNT7A. Levels of β-catenin (A) and of ERK1–2 (B, loading controls) in PC12 cells transfected with empty expression plasmid and cultured in the presence or absence of 10 mM LiCl for 24 h or transfected with a WNT7A expression plasmid.
- **Fig. 2.** PC12 cells respond specifically in a TCF reporter assay to WNT7A in transient transfection assays. A, effects of culture in the presence of 10 mM LiCl or of transfection with expression plasmids encoding WNT7A, a truncated non-functional WNT7A allele (WNT7Apx) or WNT5A on TCF-mediated transcriptional activity, employing the TCF-luciferase reporter. B, the TCF reporter response to WNT7A is dependent on the dose of transfected WNT7A expression plasmid. Increasing amounts of WNT7A expression plasmid (70–440 ng) result in increasing TCF-mediated transcriptional activity, whereas transfection of the WNT7Apx expression plasmid does not result in comparable reporter activation. The total amount of DNA transfected was maintained equivalent in all samples.

**Materials and Methods**

**Cell Culture and RNA Isolation—**PC12 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with glutamine 2 mM and 10% fetal bovine serum and maintained in a 10% CO2 humidified atmosphere. Total RNA was extracted from subconfluent monolayers as described (32).

**Western Blotting—**Western blot analysis was performed as previously described (33). Briefly, 24 h post-transfection PC12 cells were lysed in Triton X lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM sodium orthovanadate, 50 mM sodium fluoride, and 10 mM β-glycerophosphate) for 15 min. The cell lysates (PC12 cells cultured under standard conditions or in the presence of 10 mM LiCl for 24 h) were clarified by centrifugation (10,000 × g for 10 min). Protein cell lysates (80 μg) were probed using monoclonal anti-LRP5/6 (cat. 3801; Amersham Biosciences), using horseradish peroxidase-linked secondary antibodies. For the analysis of FZD expression constructs, cells were plated at a density of 2 × 106 cells/10-mm Petri dish the day before transfection. Cells were transfected using 8 μl of LipofectAMINE 2000 (Invitrogen) and 10 μg of cDNA. 72 h post-transfection, cells were rapidly rinsed in ice-cold phosphate buffered-saline and lysed for 15 min in Triton X-100 lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol) added with protease and phosphatase inhibitors. The cell lysates were clarified by centrifugation (10,000 × g for 10 min). Protein cell lysates were separated by SDS-PAGE (8% gel for LRP5 and LRP6 and 10% gel for FZD2, FZD5, and FZD7) and blotted onto nitrocellulose. Membranes were probed using monoclonal anti-LRP5/6 (cat. 3801-106; 1:100 dilution; BioVision) or polyclonal anti-pan Fzd (cat. sc-9169; 1:100 dilution; Santa Cruz Biotechnology). The immunoreactive bands were visualized by enhanced chemiluminescence (Amersham Biosciences), using horseradish peroxidase secondary antibodies.
Functional Characterization of WNT7A Signaling in PC12 Cells

\(\text{\textcopyright DNA Synthesis and RT-PCR Studies—}\) Synthesis of cDNA and RT-PCR analysis were carried out essentially as described (21, 34, 35). Briefly, human polyadenylated RNA samples from CNS and peripheral tissues were purchased from Clontech (Palo Alto, CA). cDNA was synthesized from 1 \(\mu\)l of polyadenylated RNA using Superscript II reverse transcriptase (Invitrogen) and oligo(dt) and random hexamer oligonucleotides (250 ng each) in a final volume of 20 \(\mu\)l according to the manufacturer’s instructions. Following first strand cDNA synthesis, the reaction volume was increased to 100 \(\mu\)l and 1 \(\mu\)l was used for each PCR reaction. Assuming a 50% efficiency in the reverse transcription reaction, ~5 ng of cDNA were employed in each PCR and TaqMan reaction. For the analysis of the expression of gene expression by RT-PCR, reaction conditions were 3 min at 94 \(^\circ\)C, 30 s at 94 \(^\circ\)C, then 30 s at 55 \(^\circ\)C and 30 s at 72 \(^\circ\)C for 35 cycles. For \(\beta\)-actin amplification, PCR conditions were the same as above except that the primers used were forward, 5'-TGAAACCTAAGGCAACCCGTG-3', reverse, 5'-GCTCATAGCTCTTCTCCAGG-3'. For the analysis of expression of FZD genes in PC12 cell cDNA, PCR conditions were the same as above except that the primers used were those reported previously (21). RT-PCR primers for LRP-5 and LRP-6 were as follows: LRP-5 (forward, 5'-GGACAAAGCTGTACTGCGACC-3', and reverse, 5'-TGTTGCAAGGATGGAGTGGAG-3'), and LRP-6 (forward and reverse, 5'-GCATTTTTGGGACTCTTGTTG-3').

PCR Amplification Employing a Proofreading Thermotolerant DNA Polymerase—PCR amplification was carried out employing the GeneAmp XL PCR kit, using human adult brain cDNA as a template, as described previously (21, 34, 35). Reaction conditions were according to the manufacturer’s protocol, with a final Mg(OAc) concentration of 0.8 mM. Primers sequence were as follow: forward, 5'-GGGGCGGCTATGTGTGATTGC-3'; reverse, 5'-ACAAGCTCATCCHTCTGGCCA-3' for WNT7A; forward, 5'-GGAGGAGGCACGATCAACTCA-3'; reverse, 5'-GGTTCGGGTGCAATTCTTGG-3'; for WNT5A; forward, 5'-GGGCCGCCAGGAAC-3'; reverse, 5'-GCCTCCCACACGGTGGTCT-3', for FZD2; forward, 5'-GGCGATGCTGCCGCTGGGC-3'; reverse, 5'-CTCTTACTGGCATGGACAG-3', for FZD5; forward, 5'-GGCGGAGAGCCCGCTGGC-3'; reverse, 5'-CTACCTGCGTCCTCTTGAT-3', for FZD7. PCR products were analyzed by electrophoresis on a 1% agarose gel and were automatically normalized relative to the internal standard Renilla luciferase. PCR amplification was carried out employing the GeneAmp XL PCR kit, using human adult brain cDNA as a template, as described previously (21, 34, 35). Reaction conditions were according to the manufacturer’s protocol, with a final Mg(OAc) concentration of 0.8 mM. Primers sequence were as follow: forward, 5'-GGGGCGGCTATGTGTGATTGC-3'; reverse, 5'-ACAAGCTCATCCHTCTGGCCA-3' for WNT7A; forward, 5'-GGAGGAGGCACGATCAACTCA-3'; reverse, 5'-GGTTCGGGTGCAATTCTTGG-3'; for WNT5A; forward, 5'-GGGCCGCCAGGAAC-3'; reverse, 5'-GCCTCCCACACGGTGGTCT-3', for FZD2; forward, 5'-GGCGATGCTGCCGCTGGGC-3'; reverse, 5'-CTCTTACTGGCATGGACAG-3', for FZD5; forward, 5'-GGCGGAGAGCCCGCTGGC-3'; reverse, 5'-CTACCTGCGTCCTCTTGAT-3', for FZD7.

Functional Characterization of WNT7A Signaling in PC12 Cells

- **A.** Western blot of PC12 cells and of PC12 cells transfected with pcDNA3.1-WNT7A (employing a dicistronic expression plasmid) on the activity of the TCF reporter, indicating that FZD5 is the only FZD expressed in PC12 cells that can synergize with WNT7A to induce TCF-mediated transcriptional responses.

**RESULTS**

**WNT7A Signals via the Canonical Pathway in PC12 Cells**—PC12 cells are widely employed as a neuronal cell model, are known to activate the canonical WNT pathway in response to WNT1 (39–44), and do not express WNT7A (45). We therefore selected this cell model to assay the capacity of WNT7A to activate the canonical WNT pathway. This was performed by transiently transfecting a WNT7A expression construct (35) in PC12 cells followed by an analysis of the intracellular accumu-
Functional Characterization of WNT7A Signaling in PC12 Cells

Fig. 4. FZD5, but not FZD2 or FZD7, can synergize with WNT7A to potentiate activation of the TCF reporter. Expression plasmids comprising individual FZD cDNAs or their antisense sequences were co-transfected with the WNT7A expression plasmid (thus, ligand and receptor are encoded on separate plasmids). Only the expression of FZD5 cDNA can synergize with the WNT7A expression plasmid to increase TCF-mediated transcriptional activity. A, absolute reporter activity for each sample. B, fold induction levels for panel A.

Fig. 4. FZD5, but not FZD2 or FZD7, can synergize with WNT7A to potentiate activation of the TCF reporter.

Expression plasmids comprising individual FZD cDNAs or their antisense sequences were co-transfected with the WNT7A expression plasmid (thus, ligand and receptor are encoded on separate plasmids). Only the expression of FZD5 cDNA can synergize with the WNT7A expression plasmid to increase TCF-mediated transcriptional activity. A, absolute reporter activity for each sample. B, fold induction levels for panel A.

Functional Characterization of WNT7A Signaling in PC12 Cells

Fig. 4. FZD5, but not FZD2 or FZD7, can synergize with WNT7A to potentiate activation of the TCF reporter. Expression plasmids comprising individual FZD cDNAs or their antisense sequences were co-transfected with the WNT7A expression plasmid (thus, ligand and receptor are encoded on separate plasmids). Only the expression of FZD5 cDNA can synergize with the WNT7A expression plasmid to increase TCF-mediated transcriptional activity. A, absolute reporter activity for each sample. B, fold induction levels for panel A.

FZD5 Can Function as a Component of the WNT7A Receptor Complex—WNT signaling through the canonical pathway involves the dismantling of the AXIN-APC-GSK3β macromolecular complex, stabilization of β-catenin, and stimulation of TCF-mediated transcription through the activation of a receptor complex comprised of members of the FZD and LRP protein families (2). There are indications that distinct FZD proteins display different affinities for individual WNT ligands (23, 25). Consequently, an appropriate combination of FZD-LRP proteins must be expressed in order for PC12 cells to respond to WNT7A. The expression of FZD genes in this system was therefore analyzed by RT-PCR. The results (Fig. 3A) indicate that FZD2, FZD5, and FZD7 are expressed in PC12 cells, in general agreement with a previous report where FZD expression in PC12 cells was analyzed by Northern blotting (47). We reasoned that one or more of these FZD proteins would be responsible for mediating WNT7A signaling in PC12 cells. Therefore, the complete open reading frames for FZD2, FZD5, and FZD7 were obtained and cloned into expression constructs either individually or in tandem with the WNT7A cDNA as reporter construct (data not shown), suggesting that it cannot stimulate the Ca²⁺-dependent WNT pathway in this system.

Interestingly, although WNT7A can activate the canonical pathway in PC12 cells, it is a rather inefficient inducer of a Ca²⁺-dependent Nuclear Factor of Activated T-cells (NFAT)-reporter construct (data not shown), suggesting that it cannot stimulate the Ca²⁺-dependent WNT pathway in this system.

FZD5 Can Function as a Component of the WNT7A Receptor Complex—WNT signaling through the canonical pathway involves the dismantling of the AXIN-APC-GSK3β macromolecular complex, stabilization of β-catenin, and stimulation of TCF-mediated transcription through the activation of a receptor complex comprised of members of the FZD and LRP protein families (2). There are indications that distinct FZD proteins display different affinities for individual WNT ligands (23, 25). Consequently, an appropriate combination of FZD-LRP proteins must be expressed in order for PC12 cells to respond to WNT7A. The expression of FZD genes in this system was therefore analyzed by RT-PCR. The results (Fig. 3A) indicate that FZD2, FZD5, and FZD7 are expressed in PC12 cells, in general agreement with a previous report where FZD expression in PC12 cells was analyzed by Northern blotting (47). We reasoned that one or more of these FZD proteins would be responsible for mediating WNT7A signaling in PC12 cells. Therefore, the complete open reading frames for FZD2, FZD5, and FZD7 were obtained and cloned into expression constructs either individually or in tandem with the WNT7A cDNA as
dicistronic constructs. These plasmids were first tested by Western blotting for appropriate expression of the encoded FZD proteins in PC12 cells (Fig. 3B) and then co-transfected in the TCF-luciferase reporter assay to evaluate a possible synergistic effect with WNT7A on TCF-mediated transcription, indicative of functional interaction. The results (Fig. 3C and Fig. 4) indicate that the most substantial synergy with WNT7A in the stimulation of TCF-mediated transcription is achieved by FZD5. Although a synergistic stimulation of canonical Wnt signaling by the FZD7/WNT7A combination was never observed, some activity was displayed by the FZD2/WNT7A combination in most experiments, but only when FZD2 and WNT7A were expressed from separate plasmids. On the other hand, the synergy between FZD5 and WNT7A was observed independently of whether the FZD5 and WNT7A cDNAs were encoded by the same (dicistronic) construct (Fig. 3C and also Fig. 5) or by separate expression plasmids (Fig. 4). Importantly, transfected FZD5 cDNA was incapable of stimulating the TCF reporter in the absence of co-transfected WNT7A expression plasmid. Overall, this suggests that FZD5 is the major component of the highest affinity WNT7A receptor for canonical Wnt signaling in PC12 cells.

A FZD5-LRP6 Complex Can Function as a WNT7A Receptor—Two LRPs can function as co-receptors for FZD proteins, namely LRP5 and LRP6 (16). An RT-PCR analysis revealed that only LRP6 is expressed in PC12 cells (Fig. 5A), suggesting that the WNT7A receptor complex in PC12 cells may include FZD5 and LRP6. To analyze the specificity of LRP proteins in mediating WNT7A signaling in PC12 cells, experiments were carried out in which LRP5 or LRP6 expression plasmids were co-transfected together with WNT7A-FZD receptor combinations and the TCF-luciferase reporter. Western blotting analysis demonstrated that both LRP expression plasmids were capable of driving the expression of the corresponding LRP cDNA to comparable levels (Fig. 5B). The results of the reporter studies (Fig. 5C) indicated that both LRP5 and LRP6 can synergize with WNT7A in inducing TCF-mediated transcription in PC12 cells, probably through endogenous FZD5 receptor expression. Additionally, both LRPs can synergize with exogenously provided FZD5, again confirming the functional role of FZD5 in mediating WNT7A signaling. Therefore, both LRPs can in principle facilitate WNT7A signaling, the response to this WNT family member in PC12 cells is likely mediated by a FZD5-LRP6 receptor complex.

Modulation of WNT7A Signaling by DKK Proteins—DKK proteins represent a novel family of modulators of WNT signaling (48). DKK1, in particular, has been reported to antagonize WNT signaling via direct interaction with LRP5/6, resulting in rapid LRP co-receptor removal via Kremen proteins-mediated endocytosis and consequent inhibition of WNT signaling (49). Because the activity of DKK proteins on WNT7A signaling is unknown, the effects of co-transfected expression plasmids encoding DKK1, DKK2, or DKK3 on WNT7A-mediated stimulation of the TCF-luciferase reporter was analyzed in PC12 cells. The results (Fig. 6A) indicate that DKK1 is the only tested Dickkopf family member capable of dramatic inhibition of WNT7A signaling in PC12 cells. A small but significant induction of reporter activity by DKK2 was noted, consistent with published data indicating DKK2 as a positive modulator of WNT signaling through an interaction with LRP6 (50). To analyze the specificity of DKK proteins for LRP5 and LRP6, a series of co-transfection experiments was carried out in which the viability of individual DKK proteins was examined on LRP5- or LRP6-mediated enhancement of WNT7A signaling. As illustrated in Fig. 6, B and C, DKK1 and DKK3 can inhibit the enhancement of WNT7A signaling afforded by expression of an LRP5 or LRP6 cDNA, an effect which is not observed with DKK2. In fact, DKK2 overexpression can further induce TCF-mediated transcription when co-expressed with LRP5 (but not with LRP6). These results suggest that DKK3 can also act as a negative modulator of WNT signaling. Moreover, the data indicate that DKK2 can act as a positive modulator of WNT signaling (as previously reported), and that this activity may be mediated by LRP5. As for the other expression plasmids, the amount of protein produced by the three DKK expression constructs in PC12 cells was assayed by Western blotting using commercial antibodies specific for each DKK protein. Unfortunately, we were unable to detect any signal. Because a functional response to DKK expression was observed, we believe the inability to detect DKK protein expression from the three plasmids was not because of lack of protein expression but rather because of a technical problem associated with the use of the anti-DKK antibodies.

DISCUSSION

WNT factors have been functionally classified into transforming (e.g. WNT1, WNT3A, and WNT7A) and non-transforming...
Fig. 6. DKK1 family members modulate WNT7A signaling. A, modulation of WNT7A signaling by DKK expression plasmids, measured using a TCF reporter in transient transfection assays. DKK1 is a negative modulator of WNT7A signaling, whereas DKK2 and DKK3 expression plasmids have only a marginal influence on WNT7A-mediated reporter activation. B, modulation of WNT7A signaling by DKK expression plasmids in the presence of co-transfected LRP5. In this context, co-transfection of DKK1 or DKK3 expression plasmids inhibits TCF activity induced by WNT7A and LRP5, whereas DKK2 can further stimulate WNT7A-LRP5 signaling. C, modulation of WNT7A signaling by DKK expression plasmids in the presence of co-transfected LRP6. Co-transfection of DKK1 or DKK3 expression plasmids inhibits TCF activity induced by WNT7A and LRP6. No stimulation of TCF activity by DKK2 is observed.

Functional Characterization of WNT7A Signaling in PC12 Cells

Transfected PC12 cells provide a powerful tool for the study of WNT7A. Searching for the FZD receptor subtype mediating the action of WNT7A, we found that PC12 cells expressed the transcript encoding for FZD2, FZD5, and FZD7 receptors. This is consistent with
previous reports showing that PC12 expresses FZD2 and -5 receptors (47). In the absence of a subtype-selective antagonist, the involvement of a specific receptor can be examined by knocking down or overexpressing the receptor protein. We adopted the latter strategy and examined the ability of transfected FZD2, -5, and -7 to amplify the response to WNT7A. None of the three receptors had any effect per se (i.e. without WNT7A) on TCF-mediated transcription, suggesting that either no ligands are present or that endogenous FZDs maximally respond to the WNT proteins constitutively secreted by PC12 cells. In contrast, FZD5 was able to amplify the response to co-transfected WNT7A, whereas no substantial changes were induced by FZD2 or FZD7. This suggests that (i) FZD5 is the specific receptor subtype mediating the activation of the β-catenin pathway by WNT7A in PC12 cells; (ii) the FZD5 receptors that are constitutively expressed by PC12 cells do not saturate the response to transfected WNT7A; and (iii) FZD2 and FZD7 do not mediate the activation of the β-catenin pathway by WNT7A, unless the molecules that convey the FZD specific role in mediating the action of WNT in bone formation are induced and functionally interact in developing and adult CNS tissues. FZD5 and LRP6 may function as co-receptors for WNTs in the establishment whether WNT7A, FZD5, and LRP6 are co-localized and functionally interact in developing and adult CNS neurons. Because targeted deletion of WNT7A in mice produces defects in cerebellar maturation (in addition to skeletal and urogenital abnormalities) (12, 46, 60, 61), we expect that a similar phenotype can be produced by knocking down FZD5 at critical stages of development. This cannot be addressed in FZD5 knockout mice because these animals die in utero (10.5 dpc) because of abnormal placentalization (62). The identification of FZD5 and LRP6 as WNT7A co-receptors in the CNS might gain new insights into the physiology of cerebellar development and might provide new targets for the search of molecules involved in the pathophysiology of inherited cerebellar dysfunctions.

Acknowledgments—Drs. Cristof Niehrs, Xi He, and Michail Semenov are gratefully acknowledged for the plasmid constructs encoding DKK and LRP proteins.

REFERENCES

1. Dale, T. C. (1998) Biochem. J. 329, 209–223
2. Miller, J. R. (2002) Genome Biol. 3, Reviews 301
3. Selensticker, M. J., and Behrens, J. (2000) Biochim. Biophys. Acta 1495, 168–182
4. Wodarz, A., and Nusse, R. (1998) Annu. Rev. Cell Dev. Biol. 14, 59–88
5. Behrens, J. (2000) Ann. N. Y. Acad. Sci. 910, 21–33
6. Nusse, R., and Clevers, H. (1999) Biochim. Biophys. Acta 1424, M23-M37
7. van Nort, M., and Clevers, H. (2002) Dev. Biol. 244, 1–8
8. Smalley, S. M., and Dale, T. C. (1999) Cancer Metastasis Rev. 18, 215–230
9. Patapoutian, A., and Reichardt, L. F. (2000) Curr. Opin. Neurobiol. 10, 392–399
10. Dahmen, R. P., Koch, A., Denkhaus, D., Tonn, J. C., Sorensen, N., Berthold, F., Behrens, J., Birchmeier, W., Wiestler, O. D., and Pietsch, T. (2001) Cancer Res. 61, 7039–7045
11. Gan, D. D., Reiss, K., Carrill, T., Del Valle, L., Croul, S., Giordano, A., Fishman, P., and Khalili, K. (2001) Oncogene 20, 4864–4870
12. Hall, A. C., Lucas, F. R., and Salinas, P. C. (2000) Cell 100, 525–535
13. Koch, A., Waha, A., Tonn, J. C., Sorensen, N., Berthold, F., Wolter, M., Reifenberger, J., Hartmann, W., Friedl, W., Reifenberger, G., Wiestler, O., and Pietsch, T. (2001) Int. J. Cancer 93, 444–449
14. Morris, P. J. (1999) Biol. Essays 21, 1021–1030
15. Miller, J. R., Hocking, A. M., Brown, J. D., and Moon, R. T. (1999) Oncogene 18, 7860–7872
16. Tamaki, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, F., Saint-Jeannet, J. P., and He, X. (2000) Nature 407, 530–535
17. Woodgett, J. R. (2001) Science’s STKE http://stke.sciencemag.org/content/full/oc

18. Nusse, R. (2001) Nature 411, 255–256
19. Dickinson, M. E., and McMahon, A. P. (1999) Curr. Opin. Genet. Dev. 9, 562–566
20. Gavin, B. J., McMahon, J. A., and McMahon, A. P. (1999) Genes Dev. 4, 2319–2326
21. Sala, C. F., Formenti, E., Terstappen, G. C., and Caricasole, A. (2000) Biochem. J. 344, M23–M37
22. He, X., Saint-Jeannet, J. P., Wang, Y., Nathans, J., Dawid, I., and Varmus, H. (1992) J. Cell Biol. 119, 1517–1535
23. Porfiri, E., Rubinfeld, B., Albert, I., Hovanes, K., Waterman, M., and Polakis, P. (1997) Oncogene 15, 2833–2839
24. Shackleford, G. M., Williott, K., Wang, J., and Varmus, H. E. (1993) Neuron 11,
Functional Characterization of WNT7A Signaling in PC12 Cells

865–875

45. Erdreich-Epstein, A., and Shackleford, G. M. (1998) Growth Factors 15, 149–158
46. Parr, B. A., Avery, E. J., Cygan, J. A., and McMahon, A. P. (1998) Dev. Biol. 202, 228–234
47. Chou, A. H., Zheng, S., Itsukaichi, T., and Howard, B. D. (2000) Brain Res. Mol. Brain Res. 77, 232–245
48. Zorn, A. M. (2001) Curr. Biol. 11, R592-R595
49. Mao, B., Wu, W., Davidson, G., Marhold, J., Li, M., Meecher, B. M., Delius, H., Hoppe, D., Stannek, P., Walter, C., Glinka, A., and Niehrs, C. (2002) Nature 417, 664–667
50. Brott, B. K., and Sokol, S. Y. (2002) Mol. Cell. Biol. 22, 6100–6110
51. Sheldahl, L. C., Park, M., Malbon, C. C., and Moon, R. T. (1999) Curr. Biol. 9, 695–698
52. Shimizu, H., Julius, M. A., Giarre, M., Zheng, Z., Brown, A. M., and Kitajewski, J. (1997) Cell Growth Differ. 8, 1349–1358
53. Lucas, F. R., and Salinas, P. C. (1997) Dev. Biol. 192, 31–44
54. Lucas, F. R., Goold, R. G., Gordon-Weeks, P. R., and Salinas, P. C. (1998) J. Cell Sci. 111, 1351–1361
55. Malbon, C. C., Wang, H., and Moon, R. T. (2001) Biochem. Biophys. Res. Commun. 287, 589–593
56. Winklbauer, R., Medina, A., Swain, R. K., and Steinbeisser, H. (2001) Nature 413, 856–860
57. Pinson, K. I., Brennan, J., Monkley, S., Avery, B. J., and Skarnes, W. C. (2000) Nature 407, 535–538
58. Kato, M., Patel, M. S., Levasseur, R., Lobov, I., Chang, B. H., Glass, D. A., Hartmann, C., Li, L., Hwang, T. H., Brayton, C. F., Lang, R. A., Karsenty, G., and Chan, L. (2002) J. Cell Biol. 157, 303–314
59. Krupnik, V. E., Sharp, J. D., Jiang, C., Robison, K., Chickering, T. W., Amaravadi, L., Brown, D. E., Guyot, D., Mays, G., Leiby, K., Chang, B., Duong, T., Godearl, A. D., Gearing, D. P., Sokol, S. Y., and McCarthy, S. A. (1999) Gene 238, 301–313
60. Miller, C., and Sassoon, D. A. (1998) Development 125, 3201–3211
61. Parr, B. A., and McMahon, A. P. (1995) Nature 374, 350–353
62. Ishikawa, T., Tumia, Y., Zorn, A. M., Yoshida, H., Seldin, M. F., Nishikawa, S., and Taketo, M. M. (2003) Development 128, 25–33