Independent Mutations in Mouse Vangl2 That Cause Neural Tube Defects in Looptail Mice Impair Interaction with Members of the Dishevelled Family*S

Elena Torban‡, Hui-Jun Wang, Normand Groulx, and Philippe Gros‡§

From the Department of Biochemistry, Center for Host Resistance and McGill Cancer Center, McGill University, Montreal, Quebec H3G 1Y6, Canada

Mammalian Vangl1 and Vangl2 are highly conserved membrane proteins that have evolved from a single ancestral protein Strabismus/Van Gogh found in Drosophila. Mutations in the Vangl2 gene cause a neural tube defect (raniorachischisis) characteristic of the lopetail (Lp) mouse. Studies in model organisms indicate that Vangl proteins play a key developmental role in establishing planar cell polarity (PCP) and in regulating convergent extension (CE) movements during embryogenesis. The role of Vangl1 in these processes is virtually unknown, and the molecular function of Vangl1 and Vangl2 in PCP and CE is poorly understood. Using a yeast two-hybrid system, glutathione S-transferase pull-down and co-immunoprecipitation assays, we show that both mouse Vangl1 and Vangl2 physically interact with the three members of the cytoplasmic Dishevelled (Dvl) protein family. This interaction is shown to require both the predicted cytoplasmic C-terminal half of Vangl1/2 and a portion of the Dvl protein containing PDZ and DIX domains. In addition, we show that the two known Vangl2 loss-of-function mutations identified in two independent Lp alleles associated with neural tube defects impair binding to Dvl1, Dvl2, and Dvl3. These findings suggest a molecular mechanism for the neural tube defect seen in Lp mice. Our observations indicate that Vangl1 biochemical properties parallel those of Vangl2 and that Vangl1 might, therefore, participate in PCP and CE either in concert with Vangl2 or independently of Vangl2 in discrete cell types.

Neural tube closure is a complex developmental process that takes place early during embryogenesis and is a key step in formation of the central nervous system, including spinal cord (1). In humans, failure of the neural tube to close properly results in a group of syndromes collectively known as neural tube defects (NTDs),1 which constitute the second most frequent cause of congenital abnormalities (1 in 1000 live births) (2). The cellular and molecular mechanisms underlying NTDs are very complex, poorly understood, and difficult to study in humans. Recent genetic studies of NTDs in model organisms have identified a number of genes and proteins that play critical roles in neural tube closure (1). In our laboratory, we have identified Vangl2 (Van Gogh-like, formerly “loop-tail associated protein,” Ltap) as the gene mutated in the mouse model of severe NTD known as lopetail (Lp) (3, 4). Heterozygous Lp animals (Lp+/−) are normal except for the presence of a characteristic “kinked” (looped) tail, whereas Lp homozygotes (Lp/Lp) die at mid-gestation due to craniorachischisis, a very severe NTD that is characterized by a completely open neural tube from the midbrain/hindbrain boundary to the caudal (tail) region (3–5). Two alleles have been described for the Lp mice: naturally occurring Lp (3) and a chemically induced LpH11004 (4).

Vangl2 is a 521-amino acid transmembrane (TM) protein composed of four putative TM domains in the N-terminal half. The C-terminal half is predicted to be cytoplasmic and is possibly involved in intracellular signaling and/or interaction with other proteins (3–6). Vangl2 mRNA is embryonically expressed in a number of tissues, including the neural tube immediately prior to, during, and after closure (3–5). The NTD phenotype of both Lp mutants (Lp−/−) is associated with independent missense mutations within the Vangl2 cytoplasmic domain affecting amino acid residues otherwise conserved in the protein family: S464N (Lp) and D255E (LpH11004) (3, 4). The similar phenotypic consequences of heterozygosity (−/+ ) and homozygosity (−/−) at independent Lp alleles (4) are consistent with the recessive mode of inheritance of Lp-associated NTD strongly suggest that Lp mutations behave as loss-of-function mutations in a gene dosage-sensitive pathway, signifying a role for Vangl2 as a critical regulator of neural tube formation. Vangl2 orthologs have been found in flies (Drosophila), worma (Caenorhabditis elegans), zebraﬁsh (Danio rerio), and mammals, thus deﬁning a family of evolutionarily conserved proteins (7). Interestingly, analysis of ﬁsh, mouse, and human genomes has identiﬁed two Vangl homologous genes in each species designated Vangl1 and Vangl2.

Originally cloned in Drosophila by two groups, the primordial gene was given the dual appellation Strabismus (Stbm) or Van Gogh (Vang), based on the phenotypic appearance of certain structures in fly mutants (8, 9). The gene was shown to play a role in establishing planar cell polarity (PCP), or the orientation of epithelial cells in a planar layer (10, 11). In flies, disruption of the PCP pathway through inactivation of one of several so-called “core PCP genes” results in mis-orientation of normally highly organized specific epithelial structures such as ommatidia of eye, hairs on the wing cells, bristles on the legs,

*S The on-line version of this article (available at http://www.jbc.org) contains Fig. 1S.

| This work was supported in part by a grant from the Canadian Institutes for Health Research (CIHR). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by salary awards from the CIHR.

§ To whom correspondence should be addressed: Dept. of Biochemistry, McGill University, 3655 Drummond, Rm. 907, Montreal, Quebec H3G 1Y6, Canada. Tel.: 514-398-7291; Fax: 514-398-2603; E-mail: philippe.gros@mcgill.ca.

The abbreviations used are: NTD, neural tube defect; TM, transmembrane; PCP, planar cell polarity; RT, reverse transcription; aa, amino acid(s); CE, convergent extension; CMV, cytomegalovirus; DBD, DNA binding domain; AD, activation domain; SD, synthetic medium; GST, glutathione S-transferase.
52704

Vangl2 Mutations Impair Interaction with Dishevelled Family

and others (11, 12). In addition to Stbm/Vangl, other core PCP proteins include the seven TM domain receptor Frizzled (Fz), an atypical cadherin Starry night/Flamingo (Stan/Fml), and two cytoplasmic proteins, Dishevelled (Dsh/Dvl) and Prickle (Pk) (13–17).

Extensive studies in flies have shown that the establishment of proper planar polarity relies on the formation of a multiprotein membrane complex consisting of core PCP proteins Stbm/Vangl, Pk, Dvl, and Fz. In individual cells of the fly wing, PCP proteins are initially arranged symmetrically at the apical membrane, but become asymmetrically redistributed during the establishment of PCP. A complex formed by Stbm/Vangl and Pk assumes an apical-proximal localization, whereas a Fz-Dvl complex is redirected to the apical-distal portion of the cell (18–20). This asymmetry is believed to be key in establishing planar polarity (11). At the molecular level, fly Stbm and its vertebrate ortholog Vangl2 have been shown to interact directly with cytoplasmic Dvl and Pk proteins leading to their recruitment to the membrane (6, 20–23). Although the function of Pk in PCP is not well understood, the role of Dvl has been better characterized. Dvl proteins have a modular organization typical of adaptor proteins with three structurally conserved domains, the N-terminal DIX domain, the central PDZ domain, and the C-terminal DEP domain (24–26). Dvl has been demonstrated to play key roles in both canonical Wnt and PCP pathways (reviewed in Ref. 27). The DIX domain is dispensable for canonical Wnt signaling, whereas the DEP domain is dedicated to the PCP pathway and the PDZ functions in both (25, 29). Mutations in the DEP domain interfere with Dvl translocation to the membrane (18, 30).

Recent studies in vertebrates have revealed that Vangl2 regulates the process of convergent extension (CE, controlled translocation to the membrane (18, 30). Vangl2 Mutations Impair Interaction with Dishevelled Family

Plasmodi—Total RNA from mouse embryos (E14.5) was used as a template for reverse transcriptase and polymerase chain reaction amplification (RT-PCR) of cDNAs corresponding to mouse Vangl1 and Vangl2 with Taq-HiFi Polymerase (Invitrogen), as previously described (3). cDNA products corresponding to the proposed cytoplasmic domains of mouse Vangl1 (251–526 aa) and Vangl2 (238–521 aa) were subcloned into plasmid vector pBDT7 (Clontech) to yield pBd-mVangl1 and pBd-D-mVangl2 and into plasmid pGEX4T (Amersham Biosciences) to produce pGST-mVangl1 and pGST-mVangl2. Restriction enzyme sites (in brackets and underlined in sequences) were incorporated within oligonucleotide primers for in-frame insertion of Vangl1/2 cDNAs in corresponding fusion proteins. The following oligonucleotide primers were used: pBd-mVangl1: 5′-CAAAGAAGATCCATCTACCTCGACGG- GATGTC-3′ (EcoRI) and 5′-TTCCTGGAGGTTTCCAAGTTGACT- GCAGC-3′ (BamHI); pBd-mVangl2: 5′-CAAGAGCATACTAGGAGGACTGACG- ATTACGGAATCGTCG-3′ (BamHI); and 5′-TTTCCCTTTTTCTGGTCT- GCAGC-3′ (EcoRI); pGST-mVangl1: 5′-CAAGAGGAAACTTCCTACATGATGTATCAGT- CCTACAGCAGA-3′ (EcoRI); pGST-mVangl2: 5′-CAAGAGGAATTCCTACATGATGTTTCCTGCTACAGACAGCACC-3′ (EcoRI); and 5′-TTTCCCTTTCTGGTCTGCAGC-3′ (EcoRI); pGST-mVangl2: 5′-CAAGAGGAAATTTCTCCCGCTTCCTCAGCTCAGCACC-3′ (EcoRI); and 5′-TTTCCCTTTCTGGTCTGCAGC-3′ (EcoRI); pGST-mVangl2: 5′-CAAGAGGAAATTTCTCCCGCTTCCTCAGCTCAGCACC-3′ (EcoRI); and 5′-TTTCCCTTTCTGGTCTGCAGC-3′ (EcoRI); pGST-mVangl2: 5′-CAAGAGGAAATTTCTCCCGCTTCCTCAGCTCAGCACC-3′ (EcoRI); and 5′-TTTCCCTTTCTGGTCTGCAGC-3′ (EcoRI); pGST-mVangl2: 5′-CAAGAGGAAATTTCTCCCGCTTCCTCAGCTCAGCACC-3′ (EcoRI); and 5′-TTTCCCTTTCTGGTCTGCAGC-3′ (EcoRI).

The commercially available yeast two-hybrid system Matchmaker system 3 (pK1612–1, Clontech) was used to study possible interactions between Vangl1/Vangl2 proteins and differ-

2 R. Nuse (2001). The Wnt gene homepage, www.stanford.edu/~rmuse/wntwindow.html.
Vangl2 Mutations Impair Interaction with Dishevelled Family

52705

ent domains of Dishevelled. The procedures used for these studies were exactly as described by the manufacturer (Clontech). The system is based on the use of Saccharomyces cerevisiae strain AH109, which is engineered to produce different PDZ domain proteins in yeast cells. We also used a common strain of Escherichia coli (Biology) to produce recombinant PDZ domain proteins. Our results indicate that the use of this system can be a useful tool for studying PDZ domain interactions.

Results

Vangl Protein Family—A search of public sequence databases reveals the presence of two Vangl genes (Vangl1, Vangl2) and two homologous genes (Mur mutation). The two genes are expressed in various tissues with different levels of expression. The expression of these genes is regulated by different mechanisms, including transcriptional and post-transcriptional regulation. These genes play important roles in cell adhesion, cell migration, and cell survival.

Antibody Production—Recombinant Vangl1 and Vangl2 polypeptides were purified from E. coli as GST fusions and used as immunogens to raise polyclonal rabbit sera in several different strains of mice. The sera were used in various assays, including Western blotting, immunoprecipitation, and immunofluorescence. The results showed that the antibodies recognize both Vangl1 and Vangl2 proteins, suggesting that these proteins are involved in different cellular processes.

Co-immunoprecipitation—To study the interaction between Vangl1 and Vangl2, we used a co-immunoprecipitation assay. The lysates from transiently transfected HEK293 cells (500 µl) were incubated with either 1 µg of the appropriate antibody (Vangl1, Vangl2) and 10 µg/ml of GST (Amersham Biosciences) as control. Immunoprecipitation was performed with Protein G beads, and the complexes were analyzed by Western blotting. The results showed that Vangl1 and Vangl2 proteins interact with each other in HEK293 cells, suggesting that these proteins play a role in cell adhesion and migration.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.

Hydropathy profiling and analysis of hydrophobic moments—Hydropathy profiling is a method used to predict the transmembrane topology of proteins. The hydropathy profiles of Vangl1 and Vangl2 proteins showed the presence of multiple transmembrane domains. The analysis of hydrophobic moments indicated the presence of potential amphipathic helices, suggesting that these proteins have a significant membrane association.
Material). Their functional relevance \textit{in vivo} is however uncertain, because these predicted glycosylation sites appear to be located intracellularly.

Sequence similarity is not uniformly distributed along the length of Vangl1 and Vangl2 proteins (Fig. 1). In particular, two areas near the N terminus (position 14–53, zone I) and in the cytoplasmic half (positions 310–340, zone III) show rather low degree of similarity (<40%), whereas two regions in the cytoplasmic half (zones II and IV) share >80% identity (Fig. 1B). Regions of high sequence conservation may be crucial for Vangl function. This proposition is supported by the observation that Vangl2 mutations S464N and D255E identified in \textit{looptail} (\textit{Lp}) mice are mapped to proposed zones II and IV (Fig. 1B), the regions sharing the highest degree of sequence conservation in the
Vangl protein family. These observations highlight the role of the Vangl cytoplasmic domain in transducing PCP signaling, possibly via protein-protein interactions with other PCP partners. Finally, alignment of the 9 Vangl protein sequences identifies a number of invariant residues (92/520), including 17 basic, 10 acidic residues, and 3 prolines (Supplemental Material). Included into this group are the four cysteine residues of human/mouse Vangl1/2, which suggests that they might play a critical structural (disulfide bridge) or functional role that has been conserved during evolution of Vangl proteins.

**Vangl1 and Vangl2 Proteins Interact with Mammalian Dvl Proteins—Dishevelled (Dvl) is a cytoplasmic protein involved in the Wnt/β-catenin and PCP pathways (27). It is recruited to the cell membrane in response to PCP signaling (20). The C-terminal half of the Vangl2/Stbm is sufficient to bind and recruit Dvl to the plasma membrane in frog animal cap tissues (6). Three Dvl homologs have been identified in mammals, Dvl1, Dvl2, and Dvl3, but their possible interactions with Vangl1 or Vangl2 have not been studied. To determine if the structural homologies within the Vangl protein family translate into functional similarities, we tested the ability of Vangl1 and Vangl2 to interact individually with Dvl proteins in a series of protein-protein interaction assays.

In a first set of experiments, we used a yeast two-hybrid system (Matchmaker 3.0, Clontech). Each Vangl and Dvl protein was fused to either the DNA binding domain (DBD) or the activation domain (AD) of the transcriptional activator GAL4 (Fig. 2A). Interaction between the two test partners reconstitutes GAL4 activity detectable by activation of four reporter genes within the Vangl2/Stbm, which suggests that they might play a critical structural (disulfide bridge) or functional role that has been conserved during evolution of Vangl proteins.
FIG. 2. Interactions of mouse Vangl1 and Vangl2 with Dishevelled proteins using a yeast two-hybrid system. A, schematic representation of major predicted structural features of Vangl and Dvl proteins, including TM domains (numbered 1 to 4), a PDZ-binding motif (PBM) at the C terminus, and a cytoplasmic domain of Vangl and DIX (Dishevelled-Axin), PDZ (PSD95-Discs Large-ZO1), and DEP (Dishevelled-EGL10-Pleckstrin) domains of Dvl (top). In the yeast two-hybrid system used, the cytoplasmic domains of Vangl1 (mVangl1, positions 251–526) and Vangl2 (mVangl2, 238–521) were independently fused to the DNA binding domain of GAL4 (GAL4DBD). Independent fusions to the transcriptional activation domain of GAL (GAL4AD) were generated and consisted of the N-terminal domains of Dvl1 (mDvl1-5', positions 1–404), Dvl2 (mDvl2-5', positions 1–418), and Dvl3 (mDvl3-5', positions 1–395) as well as the DEP domain containing C-terminal segment of Dvl3 (mDvl3-3', positions 389–717) and the DIX domain of Dvl1 (mDvl1-DIX, positions 1–87). B, detection of the various GAL4DBD (Vangl constructs), and GAL4AD (Dvl constructs) fusions expressed in S. cerevisiae cells. Whole cell protein extracts were separated by SDS-PAGE and analyzed by immunoblotting using monoclonal anti-c-Myc (left panel) and anti-HA (right panel) antibodies directed against epitope tags inserted in-frame in each protein. DBD indicates a control construct consisting of the DNA binding domain of GAL4 only expressed in yeast. The identity of each fusion protein is indicated on top of each blot, and their positions on the gel are indicated by arrowheads. Positions of molecular mass markers are shown. C, haploid yeast cells expressing either the Vangl GAL4DBD fusions (columns) or the various Dvl GAL4AD fusions (rows) were mated to create

Vangl2 Mutations Impair Interaction with Dishevelled Family
protein dosage, because the mutation is inherited in co-dominant fashion, e.g. heterozygosity at Lp (Lp/+) causes a phenotype (looped tail). Both mutations are in the proposed cytoplasmic domain of Vangl2 and affect residues that are either extremely conserved (Ser-464) or invariant (Asp-255) in the Vangl protein family (Fig. 1S, Supplemental Material). Because the molecular basis for the loss of Vangl2 function in Lp alleles is unknown, it was of interest to determine whether these mutations affect interaction with members of the Dvl family. For this, the S464N and D255E mutations were independently built into the cytoplasmic portion of mouse Vangl2 followed by expression in S. cerevisiae yeast cells. Immunoblots of total protein extracts from yeast transformants show that both mutants are expressed in yeast cells at levels comparable to that seen for the wild type protein (Fig. 5A). This suggests that the two mutations do not affect protein stability nor do they cause rapid degradation in yeast cells. These Vangl2 transformants were mated to tester stocks expressing different fragments of Dvl proteins; effect of mutations on Vangl2/Dvl protein interaction were monitored by plating diploids on selective media of increasing levels of stringency. Experiments in Fig. 5B demonstrate that the S464N and D255E mutations impair interaction with Dvl proteins. Under condition of medium stringency (-His/-Trp/-Leu) both mutant Vangl2 proteins...
**FIG. 4.** Reciprocal interactions between Vangl1 and Dvl2 detected by co-immunoprecipitation. **A**, sequence of Vangl1 and Vangl2 segments used to construct GST fusion immunogens to produce isoform-specific anti-Vangl1 and anti-Vangl2 rabbit polyclonal antisera; identical amino acids are *shaded*. **B**, the isoform specificity of the anti-Vangl1 and anti-Vangl2 antisera was verified by immunoblotting. Total cell extracts
lose binding to Dvl1 only, whereas under a high stringency condition (-Ade/-His/-Trp/-Leu) the mutations abrogate binding to all three Dvl proteins. These findings suggest a molecular basis for the loss of Vangl2 protein function seen in Lp mouse mutants. Considering that stringency of selection medium provides a “semi-quantitative” measure of the binding affinities between different partners, our findings further suggest that the Vangl2/Dvl1 interaction is most mutation-sensitive, possibly reflecting a weaker affinity of Vangl2 for Dvl1 than for Dvl2/3.

**DISCUSSION**

The Vangl1 and Vangl2 genes encode membrane proteins that have been highly conserved during evolution and are derived from an ancestral precursor Stbm/Vang found in flies. Genetic studies in model organisms have shown that Vangl2 plays a critical role during development (31, 33): mutations in mice cause defects in the formation of the neural tube (3–5) and Stbm/Vang inactivation in flies alters the organization of structures in the eye (ommatidia) and wing (hair) (8, 9). These phenotypes indicate that Vangl proteins function to organize planar cell polarity and to direct convergent extension (CE) movements during embryogenesis. The precise function of Vangl proteins during these complex processes is poorly understood, and the mechanism by which Vangl2 inactivation leads to neural tube defects is completely unknown.

Unlike flies and worms, which have a single Vangl gene, most studied vertebrates (except Xenopus) have two, Vangl1 and Vangl2. In a first attempt to identify structure/function relationships in this protein family, we have carried out multiple sequence alignments of known Vangl proteins to search for conserved structural features. We identified several common domains that might underlie characteristic functions of the Vangl protein family. A first conserved feature of Vangl proteins is their four putative TM domains. Interestingly, identities of TM1, -2, and -3 as transmembrane domains are predicted with a higher degree of certainty than TM4, raising the possibility that there are only three TM domains. An invariant PDZ binding motif is identified at the C terminus and presumably interacts with PDZ-containing proteins normally found at the cytoplasmic face of the plasma membrane (46, 47). The presence of multiple conserved N-linked glycosylation sites (NX/S/T) in the N-terminal domain suggests that this domain might in fact be extracellular. Taken together, an alternative model for Vangl proteins with an intracellular C terminus, three TM domains, and an extracellular N terminus might be proposed. Distinguishing between these two models will require additional direct topological studies of Vangl proteins by epitope mapping (48) or other methods (49).

A second observation is that the predicted cytoplasmic domain is the most conserved region of Vangl proteins. In particular, two sub-domains (II and IV) show a very high degree of similarity, suggesting that they serve in a conserved function of these proteins. This is highlighted by the fact that both known Lp loss-of-function mutations map to these sub-domains. These observations together with results in Figs. 2–5 strongly suggest that these two conserved subdomains play a critical role in the interaction of Vangl proteins with other core PCP proteins. Finally, we identified a number of invariant residues in addition to the two residues mutated in independent Lp alleles. There are several basic residues as well as four cysteines in mammalian Vangl1/2 that are absolutely conserved across the Vangl family. This suggests that disulfide bridges might participate in a common membrane-associated organization of these proteins.

Neural tube closure involves CE, and mutations in Vangl2 and Dvl1/Dvl2 cause the same neural tube defect called craniorachischisis (3, 4, 38). Both Vangl2 and Dvl proteins are central to the PCP and CE pathways (31, 35, 39), and they have been shown to interact physically (6, 20). However, it is unclear whether these properties extend to all members of the Vangl and Dvl families. Thus, we looked for possible interactions between mouse Vangl1/2 and Dvl1/2/3 proteins. Using a yeast two-hybrid system, GST pull-down, and co-immunoprecipitation assays, we observed that Vangl1 and Vangl2 could interact with all three Dvl proteins. This involves the cytoplasmic domain of Vangl1/2 and the N-terminal portions of Dvl (DIX plus PDZ domains) but does not require the N-terminal portion of Vangl1/2. Interestingly, the cytoplasmic domain of Stbm/Vang has been shown to interact with another PCP protein, Prickle (20, 23); in particular, an 85-amino acid segment overlapping the poorly conserved sub-domain III has been shown to underlie the Plk interaction (23). Additional studies are required to determine if interactions with Dvl and Plk involve independent or overlapping sets of Vangl1/2 C-terminal segments. Finally, the sequence-divergent N-terminal portion of Vangl proteins is nevertheless important for function, because a 13-amino acid in-frame insertion at position 21 causes a loss-of-function in the Vangl2 homolog of the zebrafish trilobite (tril(240h)) mutant (33). These results suggest that the N-terminal segment of Vangl proteins might be involved in other aspects of protein function, including membrane targeting, sorting to appropriate compartments, and/or possible interaction with other PCP proteins.

Independent mutations (S464N and D255E) in Vangl2 cause the severe neural tube defect observed in the two known allelic variants of the Lp mouse. S464N and D255E represent loss-of-function mutations, because: (a) these variants are specific to Lp chromosomes and are not found in 36 other phylogenetically distant mouse strains; (b) they affect residues that are either highly conserved (Ser-464) or invariant (Asp-255) in the Vangl family; and (c) the Lp defect can be corrected in transgenic mice carrying bacterial artificial chromosome clones containing an intact copy of Vangl2 (3, 4, 50). The NTD of Lp mouse is inherited in a recessive fashion but appears to be in a gene dosage-sensitive pathway, because the Lp/+ mouse exhibits a mild looped tail phenotype, whereas the Lp/Lp mouse has both a looped tail and severe craniorachischisis. Here we show that the two Lp mutations abrogate or strongly impair interaction between Vangl2 and all three members of the Dvl family, thereby providing a molecular basis for craniorachischisis in Lp mice. How do these results fit with the known role of Vangl and Dvl in PCP and CE? The little functional data available come from subcellular localization studies in flies during PCP signaling in the eye and in the wing (reviewed in Refs. 11 and 39). In both cases, the core PCP proteins Dsh, Plk, Stbm/Vangl, and...
FIG. 5. Loss-of-function mutations in Vangl2 impair interaction with Dvl proteins in a yeast two-hybrid system. A, fusion proteins, containing the GAL4DBD and the cytoplasmic domains of either wild type Vangl2 (Vangl2wt) or Vangl2 mutant D255E (Vangl2–255) and S464N (Vangl2–464) corresponding to independent alleles of the neural tube defect-causing looptail mouse mutation, were expressed in yeast cells and analyzed by immunoblotting with anti-c-Myc antibody. The expressed proteins are marked by arrowheads; molecular weights of the proteins markers are indicated. B, the ability of wild type and mutant variants of Vangl2 to interact with different Dvl proteins was tested in a yeast two-hybrid system, as described in legend to Fig. 2. The graded stringencies of the selection media are indicated as low, intermediate, and high below each panel.
Vangl2 Mutations Impair Interaction with Dishevelled Family

Fz cluster at the apical side of the cell at the beginning of PCP signaling and then become asymmetrically distributed at the end of signaling. Our findings suggest that Vangl2 mutations interfere with binding to Dvl proteins, implying that this physical association is critical for regulation of PCP and CE during mammalian development. It is conceivable that disruption of the Vangl2-Dvl part of the tertiary multiprotein complex would affect interactions of other PCP proteins within the complex leading to the interruption of the PCP signaling event. It will be interesting to analyze a parallel effect of Vangl2 mutations on interactions with other PCP proteins.

In this study we show that, like Vangl2, Vangl1 can interact with the three Dvl proteins, raising the interesting possibility that Vangl1 might be implicated in regulation of PCP and CE during embryogenesis. Vangl1 and Vangl2 might be functionally redundant but could regulate PCP/CE in distinct cell populations individually expressing one or the other protein. Alternatively, Vangl1 and Vangl2, although highly similar, might have certain distinguishing functional features that would be simultaneously required for directing PCP/CE in the same cells. A formal clarification of the role of Vangl1, if any, in establishing PCP and CE (including neural tube closure) awaits the creation and characterization of a mouse mutant awaiting the creation and characterization of a mouse mutant.
Independent Mutations in Mouse Vangl2 That Cause Neural Tube Defects in Looptail Mice Impair Interaction with Members of the Dishevelled Family
Elena Torban, Hui-Jun Wang, Normand Groulx and Philippe Gros

J. Biol. Chem. 2004, 279:52703-52713.
doi: 10.1074/jbc.M408675200 originally published online September 29, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M408675200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2004/10/07/M408675200.DC1

This article cites 50 references, 21 of which can be accessed free at
http://www.jbc.org/content/279/50/52703.full.html#ref-list-1