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The planar cell polarity (PCP) protein Diversin translocates to the nucleus to interact with the transcription factor AF9

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ABSTRACT

The planar cell polarity (PCP) pathway, a β-catenin-independent branch of the Wnt signaling pathway, orients cells and their appendages with respect to the body axes. Diversin, the mammalian homolog of the *Drosophila* PCP protein Diego, acts as a molecular switch that blocks β-catenin-dependent and promotes β-catenin-independent Wnt signaling. We report now that Diversin, containing several nuclear localization signals, translocates to the nucleus, where it interacts with the transcription factor AF9. Both Diversin and AF9 block canonical Wnt signaling; however, this occurs independently of each other, and does not require nuclear Diversin. In contrast, AF9 strongly augments the Diversin-driven activation of c-Jun N-terminal kinase (JNK)-dependent gene expression in the nucleus, and this augmentation largely depends on the presence of nuclear Diversin. Thus, our findings reveal that components of the PCP cascade translocate to the nucleus to participate in transcriptional regulation and PCP signaling.

Introduction

Wnt signaling cascades activate cellular programs that range from migration and proliferation to cell fate determination and stem cell renewal. These pathways enable cells to translate environmental cues into complex morphogenetic programs. In canonical Wnt signaling, binding of secreted Wnt molecules to Frizzled receptors and low-density-lipoprotein-related protein (LPR) induces phosphorylation of Dishevelled, inhibiting the β-catenin degradation complex. As a consequence, β-catenin escapes proteasomal degradation and translocates to the nucleus, where it interacts with transcription factors of the LEF/TCF family to activate Wnt-specific gene transcription. In *Drosophila*, a β-catenin-independent branch of the Wnt signaling pathway, the planar cell polarity (PCP) pathway, orients epithelial cells in the plane of a tissue (reviewed in [1,2]). By an unknown mechanism, upstream PCP components such as Fat and Dachsous trigger the asymmetric subcellular distribution of the PCP core proteins Frizzled (Fz), Dishevelled (Dvl), Flamingo/Starry Night (Fmi, Stan), Strabismus/Van Gogh (Stbm/Vang), Prickle (Pk), and Diego (Dgo). In the *Drosophila* wing, Fz, Dvl, and Dgo move to the distal side of the cell, while Pk and Stbm accumulate at the proximal plasma membrane. PCP effectors like Inturned (In), Fuzzy (Fy), and RhoA then reorganize the cytoskeleton to ensure proper cell morphogenesis (reviewed in [1,3]). However, in some tissues such as the *Drosophila* eye, normal PCP signaling requires JNK-dependent gene transcription [4,5].

Dgo, a six ankyrin repeat protein, was originally identified to localize Fmi in response to Frizzled signaling to the proximal/distal boundaries of the *Drosophila* wing [6]. Dgo is recruited to the plasma membrane by Frizzled receptors, where it interacts with Pk and Stbm/Vang through its N-terminal ankyrin repeats to maintain the apical localization of Fmi [7]. The mammalian homolog of Dgo, Diversin, interacts with Dvl, and recruits Casein kinase Iε and members of the Axin family to target β-catenin for degradation, thereby inhibiting canonical Wnt signaling [8]. Diversin is also involved in JNK activation, and plays a crucial role in zebrafish gastrulation and heart formation [9]. In the present study we describe that Diversin translocates to the nucleus, where it interacts with the putative transcriptional modulator AF9 to promote JNK-dependent gene transcription.

Materials and methods

Reagents and plasmids. Full-length human AF9 and mouse Diversin were generous gifts from C. Hemenway and W. Birchmeyer, respectively. Full-length and truncated version were created by PCR and standard cloning techniques, and fused to YFP- (eYFP-C1, Clontech), FLAG- (pcDNA6, Invitrogen), or V5-containing expression vectors (pcDNA6, Invitrogen). Antibodies used in this study included mouse M2 antibody to FLAG (Sigma), mouse antibody to V5 (Serotec), mouse antibody to GFP (MBL), rabbit

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antibody to MLLT3 (Atlas), and mouse antibody to Diversin (Nanotools). The position of amino acids are based on NM_001012450 (Diversin) or NM_004529 (AF9).

Yeast two-hybrid cDNA screening. Yeast two-hybrid screening was performed by the Genomics & Proteomics Core Facilities of the DKFZ (Heidelberg, Germany; Dr. M. Kögl) using Diversin without the ankyrin repeat domain (amino acid 270–712) as bait in combination with a human fetal brain cDNA library (Pretransformed Human Fetal Brain Matchmaker cDNA Library, Clonetech). The pGBKT7-Diversin was transformed into *Saccharomyces cerevisiae* strain AH109 (MATα). Yeast two-hybrid library screening was carried out by the yeast-mating procedure using *S. cerevisiae* Y187 (MATα) pretransformed with pACT2. After mating, clones were selected on minimal synthetic dropout medium (-Trp-Leu-His-Ade) containing 0.4 mM 3-amino-1, 2, 4-triazole (3AT). Protein–protein interactions were independently confirmed by yeast-two-hybrid analysis and determination of α-galactosidase activity.

Co-immunoprecipitation. Co-immunoprecipitation experiments were carried out as described, using transiently transfected HEK 293T cells [10]. For endogenous immunoprecipitation, three 10 cm dished of IMCD cells were pooled. For compartment-specific immunoprecipitation, soluble cytosolic and nuclear fractions were separated using the method described by Dignam [11].

Luciferase assay. HEK293T cells were seeded in 12-well plates and transiently transfected with a TOPflash luciferase reporter

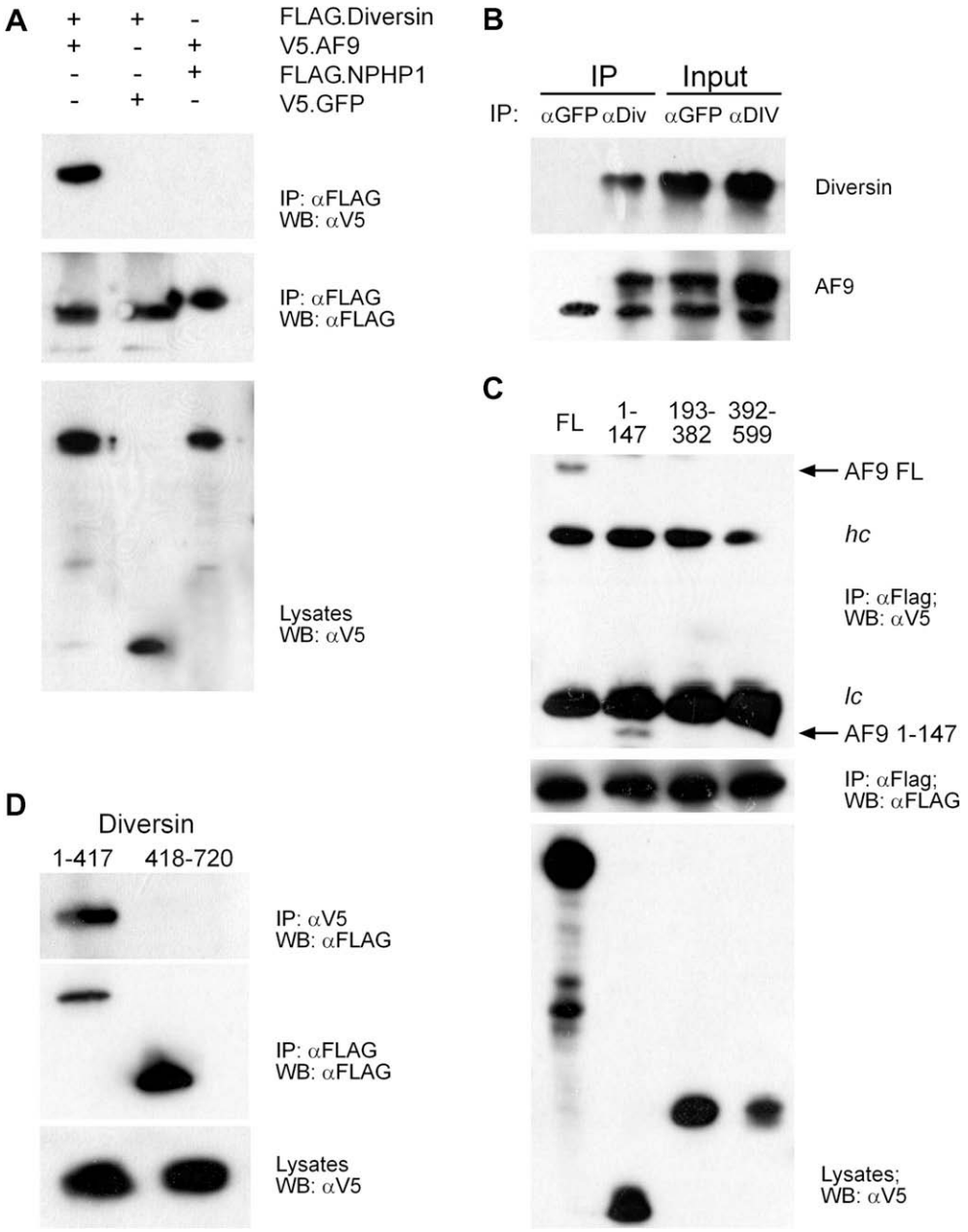

Fig. 1. The amino-terminus of Diversin interacts with AF9. (A) HEK 293T cells were transiently transfected with plasmids as indicated. Precipitated FLAG.Diversin, but not FLAG.NPHP1 immobilized V5.AF9. The control protein V5.GFP was not detectable in either precipitate. (B) Endogenous AF9 was precipitated from IMCD cells by an antibody directed against endogenous Diversin, but not by a control antibody directed against GFP. (C) FLAG9.Diversin was coexpressed with full-length and truncated forms of V5.AF9. Only full-length AF9 and the N-terminal fragment spanning amino acid 1–147 were found in immuncomplexes with Diversin. (D) Flag-tagged amino-terminal or carboxy-terminal parts of Diversin (1–417 or 418–712, respectively) were coexpressed with full-length V5-tagged AF9. Only the N-terminal fragment (spanning amino acid 1–417) interacted with full-length AF9.