Identification and characterization of Vangl2 Interactome Using Proximity-dependent Biotinylation

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Planar cell polarity (PCP) is an asymmetric organization of cells in a plane of tissue orthogonal to the apical-basal axis. Van Gogh-like protein 2 (Vangl2), a four-pass transmembrane protein, is a core protein regulating PCP signalling. Previous methods that identified Vangl2 interactome, such as yeast two-hybrids, co-immunoprecipitation and affinity-based mass spectrometry (MS), require proteins to have high binding affinity in the native condition. In order to identify proteins that interact with Vangl2 transiently or weakly, we are using a novel proximity labelling method called TurboID. TurboID is an efficient and sensitive biotin ligase that will be fused to the N-terminal of Vangl2 and biotinylate Vangl2 interacting proteins. These biotinylated proteins can therefore be pulled down and for further characterisation.

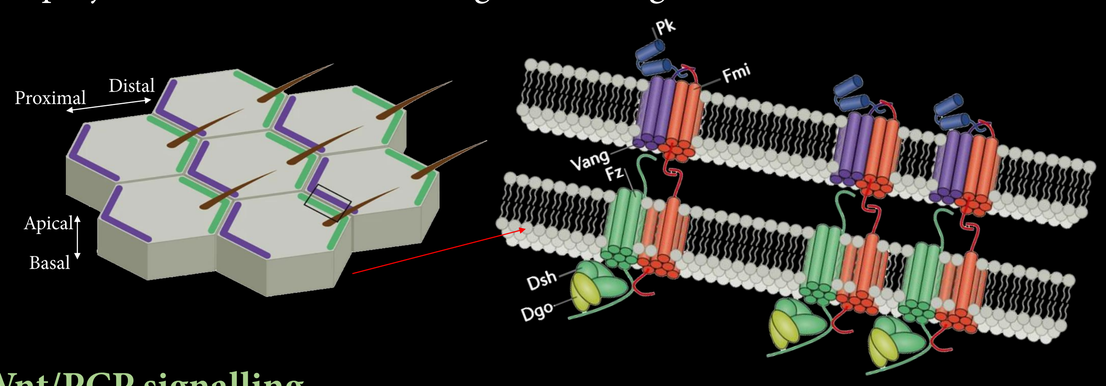
Introduction

Planar cell polarity (PCP)

A cell is polarized when its components are distributed asymmetrically. When a field of cells are polarized orthogonal to the apical-basal axis, these cells possess planar cell polarity (PCP).

Core PCP proteins

In *Drosophila,* it is suggested that there are six core proteins: Flamingo, Frizzled, Van Gogh, Dishevelled, Prickle and Diego. Vangl2 that we studied in this project is a vertebrate ortholog of Van Gogh.



Wnt/PCP signalling

In vertebrates, Wnts are the upstream molecules regulating PCP signaling, a non-canonical Wnt pathway. Comparing with canonical Wnt pathway, there is still a lack of specific readout for Wnt/PCP pathway. The objective of this project is to examine the dynamic interactome of Vangl2 with Wnt3a (canonical) and Wnt5a (non-canonical), respectively, in order to identify specific proteins that are dedicated to the Wnt/PCP pathway.

Proximity labelling (PL)

PL is a proteomic analysis method by fusing a labelling enzyme to the protein of interest which is capable of adding specific tag to the proximal proteins. Biotin ligases, which catalyze addition of biotin, can be used as labelling enzyme. Previous widely used biotin ligases, such as BioID, has limited efficiency. Recently published TurboID and miniTurbo have better performance than BioID.

Methodology

Workflow:

Generate TurboID-Vangl2, miniTurbo-Vangl2 construct

⬇

Test protein membrane localization in different cell line

⬇

Generate stable cell line expressing fused protein

⬇

Treatment cell with or without Wnt3a, Wnt5a

⬇

Treat cells with biotin to allow biotinylation

⬇

Pull-down and identify biotinylated proteins by quantitative MS

⬇

Analyse candidate list and further characterize promising candidates

Current Progress

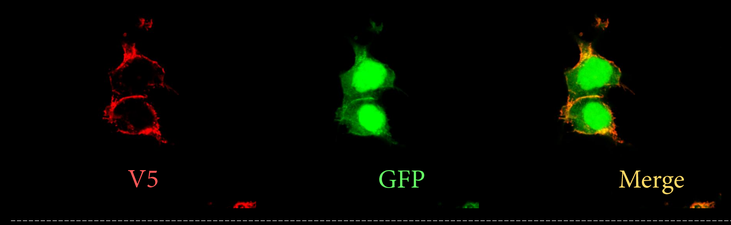
Vangl2 construct:

V5-TurboID/MiniTurbo-Vangl2 is inserted into pMSCV PIG (Addgene #21654) for transient transfection. Biotin ligase is fused at N-terminal because there is a critical function domain at C-terminal.

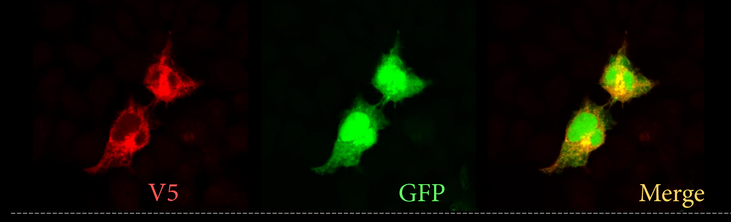
Result

Membrane localization:

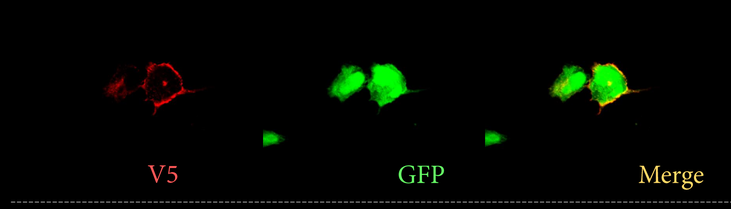
V5 (Red) indicates localization of Vangl2 fused proteins and green fluorescent protein (GFP, green) indicates cytoplasm.



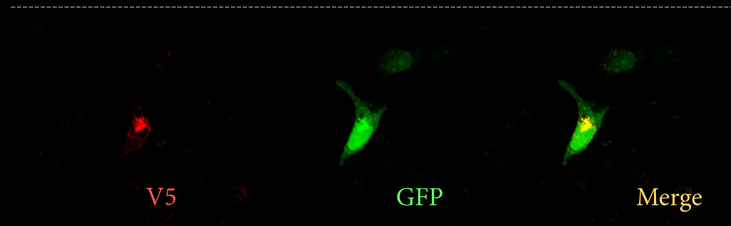
< HEK293T cell transfected with TurboID-Vangl2



< HEK293T cell transfected with MiniTurbo-Vangl2



< MDCK cell transfected with TurboID-Vangl2



< MDCK cell transfected with MiniTurbo-Vangl2

Conclusions and Future Perspectives

In both HEK293T and MDCK cells, TurboID-Vangl2 has better membrane localization than MiniTurbo-Vangl2. Therefore, in subsequent studies, TurboID-Vangl2 will be used to generate stable cell line. TurboID-Vangl2 construct is inserted into lentiviral transfer plasmid pLV-EF1a-IRES-Puro (Addgene #85132) and transfected to HEK293T cell together with packaging plasmids pD2.G and pSPAX2. After infection with lentivirus, the cells are treated with puromycin to eliminate cells without stable TurboID-Vangl2 expression. We are currenlty in the progress of generating and charactersinig the stable cell line. After obtaining stable cell lines, the proposed proximity labeling and MS will be performed.

References

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