

When It Is Hard to Get to with Genetics—Planar Cell Polarity under a Chemical Scalpel

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DOI 10.1016/j.chembiol.2011.11.005

Planar cell polarity (PCP) has been under genetic dissection for decades. More and more fundamental developmental processes have been found relying on PCP signaling. However, mechanisms of how PCP signaling generates asymmetry is still unknown. A recent paper in *Chemistry & Biology* (Sundberg et al., 2011) represents the efforts to decipher the intracellular code of polarity signaling.

Cell polarity signaling is fundamental to cellular and tissue morphogenesis. Much of our cellular and tissue organization is established by these highly conserved and versatile polarity-signaling “modules.” Cell polarity signaling components have been mostly identified by genetic approaches in invertebrates using several types of tissue morphogenesis as model systems. More and more, evidence suggests that core signaling mechanisms are shared in a variety of morphogenetic processes in both vertebrates and invertebrates. Planar cell polarity (PCP) and apical basal polarity create cellular asymmetry along two perpendicularly oriented axes in many epithelial cells and are responsible for tissue organization (Wang and Nathans, 2007).

There has been growing interest in the mechanisms of PCP signaling (Zallen, 2007). This is not only driven by the fundamental question of how cells break symmetry along the plane of tissue but also by the fascinating versatility whereby cells in all germ layers depend on planar polarity signaling. These diverse “signaling outputs” include cell fate, cell shape, migration, axon wiring, and dendrite morphology. They are more prominent in ectoderm-derived tissues, such as the skin and the nervous system. However, PCP also regulates mesoderm-derived cells, such as the elongated myoblasts and the round-shaped chondrocytes, and even endothelial cells, which form the lining of the inner surface of the blood vessels. The question of how this common set of polarity molecules create so many forms of cellular asymmetry remains open. Many developmental disorders and dysfunction of biological systems are caused by defective cell

polarity signaling and await mechanistic insights for therapeutic design.

Genetic approaches have been instrumental in identifying key PCP components. However, the lack of biochemical and cell biological insights has hindered the progress of understanding mechanisms. Recently, an elegant study, which follows up an earlier pharmacological insight with a clever chemistry trick and powerful proteomic tools, allows a better glimpse of intracellular vesicular trafficking machinery between the plasma membrane and the Golgi, which may be essential to all PCP signaling (Sundberg et al., 2011).

TNP-470 is a therapeutic derivative of the microbial metabolite, fumagillin, which is a potent inhibitor of endothelial cell proliferation and thus tumor angiogenesis. This family of compounds specifically inhibits methionine aminopeptidase-2 (MetAP-2), one of the two enzymes that remove the N-terminal Met from proteins bearing a small, uncharged amino acid at their second position. A connection between PCP signaling and endothelial proliferation during angiogenesis was made a few years ago, when TNP-470 was found to block PCP signaling but not the canonical Wnt pathway (Zhang et al., 2006). Subsequent studies demonstrated that TNP-470 treatment prevents activation of downstream effectors of noncanonical Wnt signaling, like RhoA and JNK. Following that, the Crews lab, and others, have determined that finely balanced Wnt/PCP signaling is critical for normal endothelial development; expressing a dominant-negative Dishevelled mutant, knockdown of *wnt5a* or expressing a hyperactive form of the Wnt PCP effector

DAAM1 selectively suppresses endothelial cell proliferation and impairs angiogenesis in vivo (Cheng et al., 2008; Cirone et al., 2008; Ju et al., 2010; Masckauchan et al., 2006). Despite these advances, the relevant substrate(s) of MetAP-2 in Wnt PCP signaling has not been identified.

To identify the elusive target of MetAP-2 in PCP signaling, the authors used the “N-terminal positional proteomics” strategy to narrow down candidates. MPE cells were cultured in either [¹³C₆] Arg or [¹²C₆] Arg, and the former then re-treated with TNP-470. Chemical acetylation was performed to add acetyl groups onto primary amines on N-terminal, which also resulted in acetylation of lysines’ side chains. Proteins were then trypsinized, and the internal peptides were removed by amine-capture reagent. The enriched N-terminal peptides were subject to LC-MS/MS. Rab37 was one of the proteins that showed a loss of its N-terminal methionine in the absence of TNP-470 but not in the presence of this MetAP-2 inhibitor. The authors then went on to show that Rab37 point mutation, which is resistant to MetAP-2 processes and accumulates aberrantly, phenocopies the inhibitory effects fumagillin/TNP-470 has on Wnt PCP signaling-dependent endothelial cell proliferation/network formation and convergent extension and vascularization of zebrafish embryos.

Rab37 is an under-characterized Rab, and there has been some controversy around its localization and function. It has been reported to be associated with exocytosis as well as being in the compartment between ER and Golgi. The authors then performed careful colocalization experiments and found that Rab37 is present in peri-nuclear compartment

closely associated with the *Gogli* resident protein Giantin in human umbilical vein endothelial cells. A mutant form of Rab37 (T43N), which is stabilized at GDP bound state, was detected on plasma membrane, suggesting that Rab37 is involved in vesicular trafficking from the plasma membrane to the *Golgi* apparatus.

In addition to following the lead of a drug target and using clever chemistry coupled with a new powerful tool, proteomics, the authors also used an elegant cell-cell interaction assay. They observed the organization of endothelial cells into coordinated tubular networks when cultured on Matrigel basement membrane, a quantitative in vitro proxy of angiogenesis and PCP signaling, which depends on proper levels of Rab37. This type of sensitive in vitro assay offers great opportunity to test the function of signaling components of complex molecular signaling system in specific morphogenetic processes.

The insights resulting from this study also lead to many more open questions that will further enrich our understanding of PCP signaling mechanisms. In the rescue experiments, a truncated Dvl2, which selectively activates PCP signaling, rescues endothelial cells from TNP-470-induced growth arrest. Does this suggest that Dvl2 and Rab37 are in parallel or alternative pathways? Does this mean that Dvl2 is downstream of Rab37? Additionally, levels of Rabs may be a new way of regulating these small

GTPases. A recent study shows that acetylated Met are recognized by E3 ligase (Hwang et al., 2010). Therefore, the level of Rab37 may be controlled by the ubiquitination proteasome system (UPS). There has been evidence that UPS is required for PCP signaling (Narimatsu et al., 2009). This may be an interesting connection, suggesting that an abundance of some proteins, including Rab37, may be under the regulation of the UPS system during PCP signaling. Finally, another question is the role of Rab37 in trafficking. Is it part of the active signaling process or a way to regulate the levels of certain PCP components because its own level appears to be essential to normal PCP function?

This study is very timely in this fast-moving field of PCP signaling. Endocytosis has only recently been shown to be necessary for PCP signaling (Sato et al., 2010; Yu et al., 2007) and based on the new results reported here, Rab37 may be involved in the trafficking of the endocytosed vesicles. In addition, intracellular membrane trafficking is recognized as essential, and several other Rabs have been recently implicated in various aspects of PCP signaling and function (Rab 11, Rab4, Rab23, XRab40, and Rab5). Given the multiple numbers of Rabs involved in PCP signaling, membrane trafficking may be essential in many aspects of PCP signaling, which promises to be a very active area in cell polarity research in the coming years.

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