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SPOTLIGHT

Letting go: Dishevelled phase separation recruits Axin to stabilize β-catenin

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Dishevelled exerts a molecular force that guides cell fate, but how it does so remains enigmatic. In this issue, Kang et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202205069) show Dvl2 undergoes liquid–liquid phase separation to stabilize β-catenin by pulling Axin into its biomolecular condensate at the plasma membrane.

Several critical signaling pathways control cellular proliferation, migration, and polarization. One pathway widely recognized for its influence over these processes in both normal development and pathophysiology is the Wnt/β-catenin signaling pathway (1). Within this pathway, Dishevelled interprets and propagates signals from 19 Wnts engaging 10 Frizzled (Fzd) receptors/co-receptors embedded in the plasma membrane (PM). For years, interest in how Dishevelled receives, interprets, and relays this information has been immense. Dishevelled exerts regulatory roles in various quintessential developmental steps spanning *Drosophila* to humans (Dvl; 2). If Dvl function is compromised, a range of pathologies may ensue including gross alterations in body axis formation, dysregulated skeletal and vertebrae formation, stunted hippocampal dendritic arborization, and defective cardiac and cochlear development. Hence, Dvl exerts an undeniable force in developmental biology and tumorigenesis. Despite its importance, our understanding of Dvl regulation and how it propagates downstream signals has remained limited. Kang et al. (3) now beautifully reveal a key mechanism by which Dvl2 is regulated at the PM.

In the absence of Wnt ligands, β-catenin is degraded via a destruction complex (DC) consisting of two core tumor suppressors that serve as scaffold proteins (APC and Axin) and two kinases (glycogen synthase kinase-3 β [GSK3β] and casein kinase 1 [CK1]) (4). The kinases phosphorylate β-catenin, leading to its ubiquitin-mediated proteasomal degradation. To activate canonical signaling, Wnt ligands bind to a Fzd receptor/co-receptor complex to initiate signaling at the PM. This Wnt/receptor complex recruits Dvl to Fzd where Dvl serves as a binding partner for Axin. Axin recruitment to the PM represents a key step in β-catenin stabilization, which ultimately leads to β-catenin nuclear translocation to act as a coactivator of transcription with T cell factor/lymphoid enhancer-binding factor (TCF/LEF) at Wnt target genes.

Dvl is critical for controlling the β-catenin DC. Elegant studies have demonstrated Dvl-mediated formation of a membrane proximal "signalosome," which enables critical protein interactions at the PM (5). Yet, how Dvl disrupts the β-catenin DC and promotes assembly of the signalosome remained unclear. Recent studies found Axin (6) and the DC (7) undergo liquid–liquid phase separation (LLPS) and drive DC assembly. Early evidence also suggested Dvl2 may phase separate via the DIX domain, since it was important for Dvl puncta formation (8). Wnt stimulation of endogenous Dvl also promoted Dvl2 oligomerization on the PM, where it was suggested to be regulated by its binding affinity for Fzd (9). Others have speculated Axin-Dvl heteropolymerization may disrupt Axin-Axin and Axin-APC complexes in the DC, though the mechanism by which Dvl couples the assembly of the signalosome to the disassembly of the DC remained perplexing. To address this crucial unknown, Kang et al. analyzed the highly conserved domains of Dvl, such as the DIX, PDZ, and DEP domains, as well as regions of Dvl referred to as intrinsically disordered regions (IDRs). IDRs in other proteins have been linked with intriguing biology (10), including LLPS (11), but their role in Dvl proteins has remained hidden. Using in vitro, cell-based, biophysical, microscopy-based, and transcriptional assays, the authors provide fresh new insights.

To start, CRISPR knock-in models studied the localization of mEGFP-tagged Dvl2. Confocal and grazing incidence structured illumination microscopy revealed diffuse Dvl2-mEGFP in the cytoplasm with one to two large and stable puncta with small punctate structures ranging from 0.2 to 0.5 µm in response to Wnt3a stimulation (Fig. 1). As a complementary approach, the authors expressed EGFP-tagged Dvl2 in cells in which all three Dvl paralogs were knocked out. Compared to endogenous levels, Dvl2-EGFP expression doubled and formed larger punctate structures with higher mobility. Polyethylene glycol further induced Dvl2 droplet formation and LLPS, which was sensitive to protein concentration and salt concentration. These cell-based and in vitro experiments revealed Dvl2

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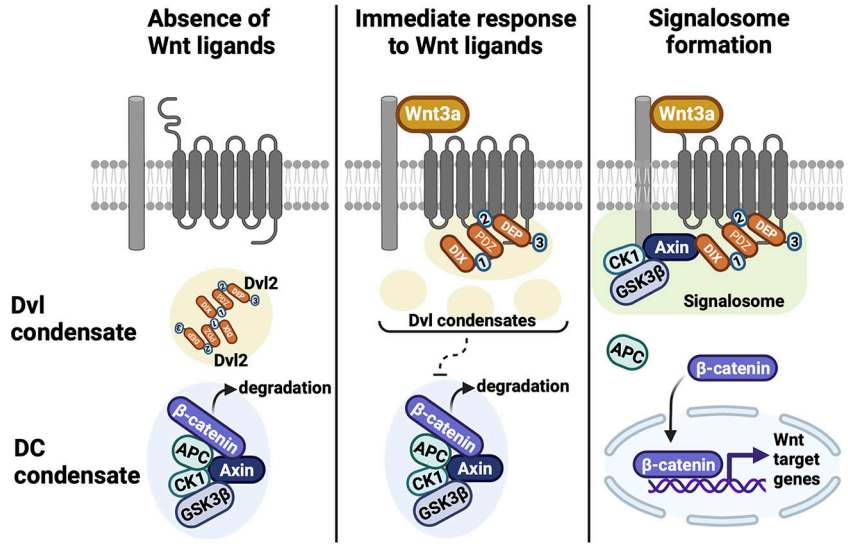

Figure 1. **Dvl LLPS induces Axin recruitment and signalosome formation upon Wnt ligand binding to promote Wnt/β-catenin signaling.** In the absence of Wnt-ligand signaling, Dvl2 forms polymerized condensates via the IDR1 (denoted as number 1 in the Dvl structure) and DIX domains, while the β-catenin DC phosphorylates and degrades β-catenin. Following stimulation with Wnt3a ligand, Dvl2 is recruited to the Fzd receptor at the PM and reduces Axin-DC condensate organization stability and function to degrade β-catenin. Following stable Dvl2 recruitment at the PM, the Dvl2 IDR1 region with the DIX domain recruits the Axin complex to the PM, resulting in complete destabilization of APC and β-catenin from the DC, forming the signalosome. The resulting unphosphorylated β-catenin translocates to the nucleus to induce canonical Wnt target gene expression. Figure created with BioRender.com.

phase separates. To identify the Dvl2 region critical for LLPS, deletion mutants of the DIX domain, as well as three non-contiguous stretches of charged amino acids (aa's) spanning two arginine-rich domains and one glutamate-rich domain within the IDR1 reduced or prevented puncta formation and droplet formation, respectively. Collectively, these studies provide major new insights that IDR1, particularly the charged residues in IDR1, mediates Dvl2 LLPS, which is further facilitated by the DIX domain (Fig. 1).

To visualize recruitment of Dvl2 to the PM receptor complex, total internal reflection fluorescence structured illumination microscopy indicated endogenous Dvl2 puncta on the PM, whose number and intensity was enhanced upon Wnt3a treatment (Fig. 1). Signalosome assembly was traced in cells expressing tagged Dvl2 and tdTomato knock-in Axin. Wnt3a rapidly increased PM-associated Dvl2 and slowly increased PM-associated Axin1. Mathematical modeling proposed stronger binding affinity of Dvl for Fzd compared to Axin. Additionally, the intracellular domains of Fzd5 and LRP6, as well as Dvl2, but not Dvl2 mutants lacking the charged IDR1 residues, promoted Dvl2 droplet formation. Collectively, Dvl2 LLPS is critical to organize droplet formation and reflects the assembly of the signalosome in vitro.

Next, the authors assessed whether Dvl2 coaxes Axin to let go of the DC and relocate to the signalosome. In a remarkable set of experiments, Dvl2 enhanced recruitment of Axin1 under Wnt stimulation, resulting in signalosome formation, while deletion of the 17 charged residues did not affect Axin1 recruitment. Upon Wnt3a stimulation, Dvl2 (but not the 17aa Dvl2 deletion mutant) reduced the size and intensity of Axin1 puncta in the cytoplasm, indicating Dvl2 suppresses Axin1 condensate formation via LLPS. *Drosophila* APC (dAPC2) also promoted Axin1 LLPS, while Dvl2 had the opposite effect, which was in part dependent on the charged aa's in IDR1. This suggests Dvl2 phase separation attenuates Axin1 droplet formation. Moreover, Dvl2 reduced the number and size of droplet formation consisting of five components (Axin1, dAPC2, GSK3β, CK1α, and β-catenin) as well as the number and intensity of Axin1 puncta containing β-catenin. Thus, Dvl2 disrupts the organization of the DC by reducing Axin1 LLPS and attenuating recruitment of other DC components into the Axin1 condensates. Most striking of all, phosphorylation of β-catenin at four aa's targeted by GSK3β and CK1α, which marks β-catenin for degradation, was dependent upon Dvl2 phase separation and the charged IDR1 residues. Finally, the authors assessed the impact of mutants that control Dvl2 phase separation (including the patch of charged aa's) via Wnt3a-induced luciferase expression. Remarkably, these results suggest the LLPS property of Dvl2 is essential to mediate Wnt/β-catenin signaling culminating in TCF/LEF reporter activation (Fig. 1).

This important study shows Dvl2 undergoes LLPS, which promotes assembly of signalosome condensates while also disrupting DC condensates. These breakthroughs lead to new questions, such as how Dvl post-translational modifications (PTMs) influence its propensity to undergo LLPS. For example, Dvl1 is acetylated at key lysines, two of which regulate its nuclear translocation and binding to gene promoters (12). Dvl is also phosphorylated (13), yet the overall function of Dvl PTMs across all paralogs remain poorly understood. Future studies should investigate the relative impact of Dvl PTMs across all domains and determine whether key residues within IDR1 play a dominant role in LLPS. Finally, while the focus of this study was Dvl2, Dvl paralogs exhibit divergent functions (13, 14). It will be important to establish the role of LLPS for the other two paralogs. As Dvl phase separates, it reminds us that letting go can often be the hardest part.

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