Wnt signalling sees spots

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The organization of signalling complexes represents the coalescence of cell biology and signal transduction. Recent work on Dishevelled (Dvl), a multifunctional component of the Wnt–Frizzled (Fz) signalling pathway, ascribes vesicle- and actin-binding properties to a single domain within the protein. This could represent a critical point for divergence of the Wnt signalling pathway.

The Dvl protein is an essential component of a ubiquitous cell communication system, the Wnt–Frizzled (Fz) pathway, which is used to regulate morphogenetic events, transcription, and ultimately, cell-fate decisions. Dvl is also an essential regulator of planar cell polarity (PCP) and can control morphological changes within the plane of epithelial tissues. This function has important parallel roles during convergent-extension movements in vertebrate gastrulation[1].

One of the most intriguing aspects of Wnt–Fz signalling is the use of different pathways to achieve different phenotypic outcomes. Cell-fate choices involve the activation of β-catenin by the Wnt–Fz pathway. β-catenin can interact with TCF in the nucleus to control transcription, and hence phenotypic outcome. However, during morphogenesis, there is no obvious role for changes in transcription. In these situations, the Wnt/Fz pathway generates cytoskeletal changes as a consequence of RhoA activation (Fig. 1).

Dvl functions downstream of the Fz receptor in both cell-fate and PCP signalling, and through an unknown mechanism, it can toggle between these two alternatives. The Dvl protein consists of several distinct modules, including a DIX, a PDZ and a DEP domain. Interestingly, in the *Drosophila melanogaster* Dvl protein, Dishevelled (Dsh), specific mutations have allowed us to allocate the cell fate and morphogenetic functions to different domains within the protein. Mutations in the DEP domain affect the PCP pathway, whereas mutations in the DIX domain affect cell-fate signalling[2,3,4]. The mechanism by which these domains control the downstream events is unclear, nor is there a good understanding of the upstream requirements (at the level of the receptor), which tell Dvl how to switch between the two outcomes. In efforts to elucidate how Dvl completes this switch, numerous Dvl binding partners have been identified, including specialized proteins, such as Daam1 (Ref. 5) and Prickled[6]. The structure of the complete Dvl protein is unknown, but there is structural information for the DEP and the PDZ portions[7,8]. As might be expected for such a multifunctional protein, Dvl has been localized to several sites within cells.

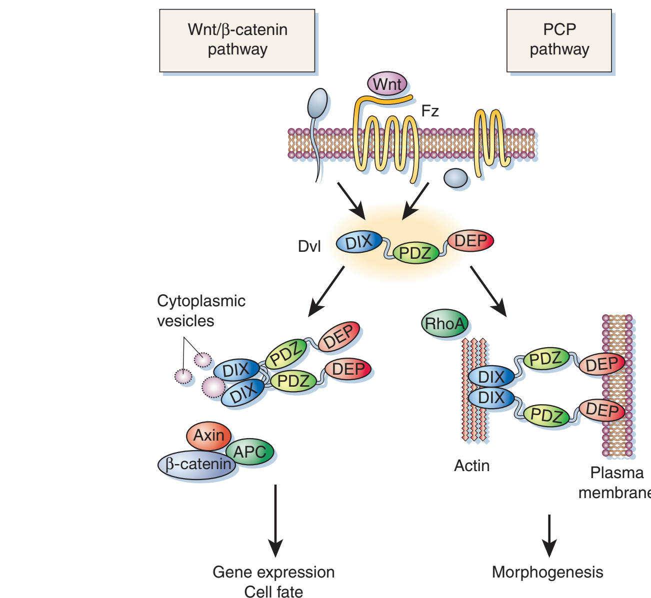

Figure 1 **Dvl has distinct functions. Dvl, which can form a homodimer, is differentially targeted to participate in either Wnt/β-catenin or PCP signalling. For its function in the determination of cell fate, Dvl is activated after binding of Wnt to the Fz receptor. New work by Capelluto *et al.* suggests that this branch of the pathway either targets a pool of vesicular Dvl or results in the association of Dvl with a subset of cytoplasmic vesicles. Dvl then triggers the accumulation of the transcriptional co-activator β-catenin. Cell-fate decisions result from β-catenin-mediated changes in gene expression. To date, no ligand has been found for PCP signalling in *Drosophila*, but the convergent–extension movements that occur during vertebrate gastrulation do require a Wnt ligand. Activation of the PCP pathway targets Dvl to the plasma membrane and results in the activation of RhoA, which effects morphological changes. The study by Capelluto *et al.* shows that Dvl interacts directly with actin and that this activity is dispensable for Wnt/β-catenin signalling. Although no role was demonstrated for actin binding in the PCP pathway, it is intriguing to consider that this function might be involved in this branch of the pathway, as it provides a direct connection between signalling and cytoskeletal changes.**

With the exception of the DEP domain, which is important for PCP signalling and localization of Dvl to the plasma membrane[2,9], it is unclear how the localization of Dvl affects its function. A study published recently in *Nature* enhances our understanding of the complex interactions in which Dvl is involved[10].

Capelluto *et al.*[10] build on the observation that Dvl localizes to small cytoplasmic vesicles and also to a subset of actin stress fibres[2,11,12]. Using nuclear magnetic resonance spectroscopy (NMR), Capelluto *et al.* now demonstrate that the vesicle- and actin-binding activities are intrinsic to the DIX domain. In addition, they determined

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that this domain is predominantly α-helical and dimeric in solution.

Using an NMR assay, they found a stretch of six amino acids located between two helices that interacts with a phospholipid mimetic, dodecylphosphocholate (DPC). This interaction was critical for vesicle association *in vivo*: mutating two key residues within the element resulted in a protein that only associated with actin stress fibres. Mutation of this vesicle-binding region rendered Dvl incapable of effecting cell-fate decisions through the Wnt/β-catenin pathway. Therefore the pool of Dvl that is localized to small intracellular vesicles may be dedicated to Wnt/β-catenin signalling.

Given the importance of the vesicle-binding element within the DIX domain, it is interesting to note that in a recent screen for signalling mutations in Dsh, the DIX domain was a hot-spot[3]. Of the seven mutations found in the DIX domain, one is located in a key amino acid of the vesicle-binding motif characterized by Capulleto *et al.* Another is located in an amino acid that would be predicted to also disrupt vesicle binding. Both of these mutations render Dvl unable to elicit cell-fate decisions, supporting the idea that the vesicle-binding activity is critical for this function.

NMR analysis in the presence of actin revealed to Capelluto *et al.* a small region that directly interacts with actin. The six-amino-acid element is similar in character and length to known actin-binding motifs. Mutating a key conserved residue within this element abrogates localization to stress fibres, but leaves the ability to activate the Wnt/β-catenin pathway intact. It remains to be seen if this mutation affects the function of Dvl in the PCP pathway.

Interestingly, the actin-binding motif abuts the vesicle-binding motif, suggesting that one activity could impede the other. Indeed, when competition experiments were performed, it was found that binding at one site precludes binding at the other. The fact that Dvl was a more potent activator of Wnt/β-catenin signalling in the absence of actin binding supports the supposition that there are distinct pools of Dvl. Thus, these two neighbouring elements within the DIX domain partition Dvl into two pools and have functions that may be mutually exclusive. However, it is unclear whether these pools are preformed, or whether signalling directs Dvl into separate pathways.

Given the evidence that the actin- and vesicle-binding sites compete, one remaining question concerns what causes one site to be used at the expense of the other. One intriguing possibility is that phosphorylation could direct Dvl down a specific pathway.

The role of Dvl phosphorylation in Wnt/β-catenin and PCP signalling has been difficult to understand. Correlations between phosphorylation, and both PCP[3,9] and Wnt/β-catenin signalling[11] have previously been demonstrated. Although no Dvl kinase has been identified for the PCP pathway, at least two kinases are required for the Wnt/β-catenin pathway[13,14,15]. Capelluto *et al.* strengthen the hypothesis that phosphorylation of Dvl is important for Wnt/β-catenin signalling with the finding that Dvl that is no longer localized to vesicles is not phosphorylated. That mutant is also unable to affect cell-fate decisions. Taken together with the dynamic nature of Dvl phosphorylation, it is probable that this modification is important in both signalling pathways[3,9,11]. Furthermore, it is tempting to speculate that different conformational states induced by differential phosphorylation could ultimately direct Dvl down one pathway or another.

The work by Capelluto *et al.* identifies motifs in the DIX domain that are responsible for localizing Dvl to compartments within the cell. It also demonstrates that one of these compartments, intracellular vesicles, is potentially the focus of Wnt/β-catenin signalling. Previous studies have shown the DEP domain of Dvl is responsible for its Fz-dependent association with the plasma membrane, an activity that is critical for PCP signalling but is dispensable for Wnt/β-catenin signalling[2,9]. An important outcome of PCP signalling is the reorganization of actin. As actin binding was not critical for Wnt/β-catenin signalling, this function of the DIX domain may be utilized in PCP signalling. An attractive hypothesis is that Dvl could couple plasma membrane targeting with the local reorganization of actin during PCP signalling.

Future studies should examine how this intracellular vesicular pool of Dvl is related to the binding of the Wnt ligand at the plasma membrane and what the nature of these Dvl-containing vesicles is. Dvl is localized to a particular subset of vesicles and it will be important to determine how this is accomplished and what the specific function of these vesicles is in Wnt/β-catenin signalling. Also of interest is whether Dvl cycles between actin and cytoplasmic vesicles and, if so, what events govern its trafficking. □

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