When you are Dishevelled, fat is good and acid is bad!

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Frizzled receptors regulate cell fate decisions and planar cell polarity by means of distinct intracellular effectors. The choice between these two signalling outputs may involve a pH-dependent interaction between Dishevelled and negatively charged lipids at the plasma membrane.

During development, cells elicit a wide range of specific biological responses to a limited repertoire of extracellular signals that are received by a similarly limited number of receptors. This obviously raises the question of how specificity arises. With regard to extracellular signals of the Wnt family, specificity is in part brought about through interaction with distinct receptors that can act in combinatorial manner (see the Wnt Homepage at http://www.stanford.edu/~rnusse/wntwindow.html). This is, however, only part of the answer, because Wnt receptors of the Frizzled (Fz) family are able to trigger distinct cellular responses on activation: Fz receptors regulate cell fate decisions by means of the 'canonical' Wnt–Fz/β-catenin pathway and planar cell polarity (PCP) via the 'non-canonical' Fz/PCP pathway. Thus, additional mechanisms must contribute to specificity. In this issue, an elegant study by Marek Mlodzik and colleagues demonstrates that Dishevelled (Dsh), a peripheral membrane protein involved in Fz signal transduction, interacts with charged lipids in a pH-dependent manner and that this interaction is essential for the PCP branch of the Fz signalling network[1]. These findings raise the novel possibility that local heterogeneities in intracellular pH modulate the output of Fz receptor activation.

Given that specific *dsh* mutations disrupt Fz/PCP signalling with no significant effect on Wnt–Fz/β-catenin signalling, it has been suggested that Dsh contributes to specificity in the cell's response to Fz activation. Dsh is a conserved multidomain protein that contains an amino-terminal DIX domain, a central PDZ domain and a DEP domain[2]. Dsh localizes at the plasma membrane in response to Fz activation, and its membrane localization is important for both Wnt–Fz/β-catenin and Fz/PCP signalling. Dsh has no known catalytic activity and probably acts by interacting with and increasing the local concentration of distinct molecular effectors at the plasma membrane. The observation that the two regions of Fz involved in a direct physical interaction with Dsh are similarly required for Wnt–Fz/β-catenin and Fz/PCP signalling argues against the view that specificity might arise from two distinct modes of interaction with Fz[3]. Alternatively, specificity may result from distinct modes of interaction of Dsh with lipids on its recruitment at the plasma membrane. Consistent with this hypothesis, the DEP domain is predicted to interact with lipids[4], and a missense mutation within the DEP domain, which results in PCP defects, causes a significant decrease in the membrane localization of Dsh[5]. Mlodzik and colleagues now show that the DEP domain interacts with negatively charged lipids. This interaction depends on proper regulation of intracellular pH and is essential for PCP signaling but not Wnt–Fz/β-catenin signalling[1].

To identify new genes required for Fz-dependent membrane localization of Dsh in *Drosophila* S2 cells, the authors conducted a genome-wide RNA-mediated interference (RNAi) screen and found that loss of Nhe2, a putative Na^+/H^+ exchanger, impaired Dsh localization. Because Na^+/H^+ exchangers regulate pH homeostasis, the authors tested whether transient intracellular acidification had similar effects on Dsh. Lowering the intracellular pH from 7.45 to 7.1 in S2 cells decreased the membrane localization of Dsh despite the presence of Fz at the plasma membrane. Although the authors did not examine whether decreased Nhe2 activity resulted in a low intracellular pH, these results suggest that membrane localization of Dsh is pH dependent. Localization of Dsh at the membrane may also be pH dependent in mammals because RNAi against Nhe3, the human homologue of Nhe2, similarly impaired the Fz-dependent recruitment of Dsh at the membrane of HEK293T cells.

Because pH-dependent interactions between peripheral membrane proteins and the negatively charged cytoplasmic side of the plasma membrane often involve a positively charged surface in proteins, the authors tested the importance of a cluster of six basic residues present at the surface of the DEP domain of Dsh for lipid binding. First, the DEP domain was shown to interact *in vitro* with negatively charged small unilamellar vesicles (SUVs) but not with neutral SUVs. Second, this interaction was abolished on the replacement of five basic amino acids of this cluster with glutamate residues. Third, the introduction of these KR/E[5×] mutations into full-length Dsh blocked the Fz-dependent localization of Dsh at the plasma membrane in cultured cells. Decreasing the negative charge on the inner surface of the plasma membrane by treating cells with sphingosine, a cationic lipid, rescued this defect. Together, these results clearly indicate that the DEP domain interacts with negatively charged lipids and that this interaction is important for the Fz-dependent localization of Dsh (Fig. 1).

The authors then addressed whether this interaction is important for the activity of Dsh *in vivo*. They first attempted to study the function of Nhe2 *in vivo* but unfortunately this proved to be challenging because a complete loss of Nhe2 activity resulted in cell lethality. To circumvent this difficulty, the authors used RNAi to reduce the level of expression of Nhe2 in a stage-specific and tissue-specific manner. Partial loss of Nhe2 activity in the eye resulted in morphological defects that included weak PCP defects. Additionally, the eye PCP phenotype resulting from increased Fz expression was suppressed by decreasing the level of Nhe2 activity. Although these genetic data are entirely consistent with the notion that Nhe2 regulates the activity of Dsh, their interpretation is complicated by Nhe2 functional pleiotropy, and several issues could not easily be addressed. For example, it was not possible to test directly whether the localization of Dsh *in vivo* is affected by a decrease in Nhe2 activity and whether Nhe2-dependent defects are associated with reduced intracellular pH.

Because genetic analysis of the Nhe2 gene did not unambiguously demonstrate a role for the intracellular pH in Dsh localization,

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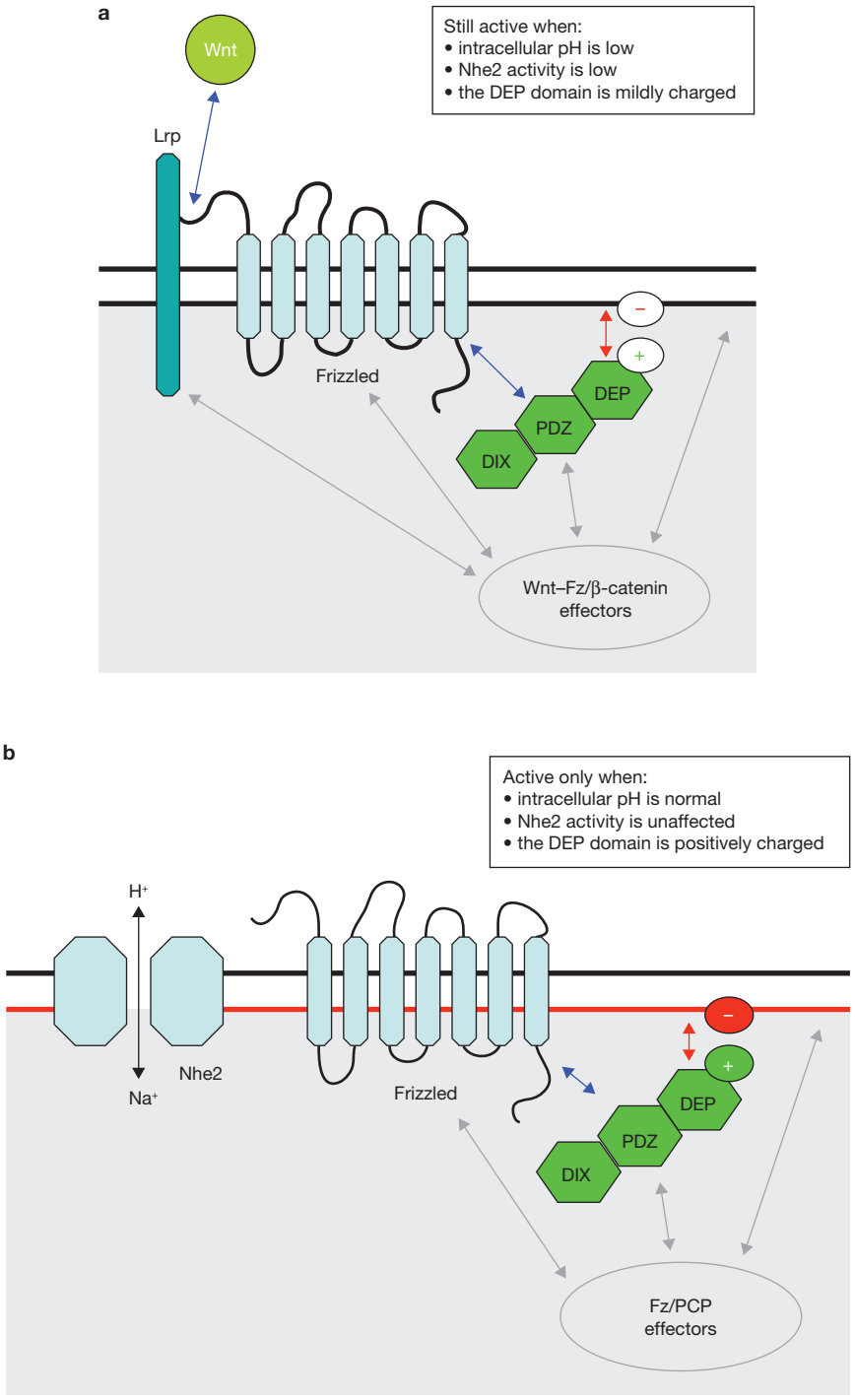

Figure 1 The two branches of Fz receptor signalling function differently. The three domains of the Dsh protein (dark green) are indicated. Molecular interactions are indicated by arrows. The interaction between the positively charged DEP domain and the negatively charged membrane is indicated by a red arrow. (**a**) Wnt–Fz/β-catenin signalling. Transient localization of Dsh at the plasma membrane may be sufficient to activate Wnt–Fz/β-catenin signalling on the Wnt-mediated activation of Fz. In other words, there is no need for a strong interaction between the DEP domain and the inner face of the plasma membrane: signalling can still occur when Nhe2 activity is reduced and/or when the number of positive charges at the surface of the DEP domain is decreased. (**b**) Fz/PCP signalling. Stable localization of Dsh at the plasma membrane may be required for PCP. Fz/PCP signalling cannot occur if the intracellular pH is lower than normal, when Nhe2 activity is reduced and/or when the number of positive charges at the surface of the DEP domain is decreased. The inner face of the plasma membrane is coloured red to indicate a high concentration of negative charges associated with either high pH values and/or a high concentration of negatively charged lipids.

the authors examined the role *in vivo* of the polybasic cluster of the DEP domain. Using a functional rescue assay, they found that expression of Dsh carrying the KR/E[5×] mutations that prevented its membrane localization in cultured cells failed to rescue the lethality associated with a strong *dsh* mutation. This indicates that the polybasic cluster is essential for the Wnt–Fz/β-catenin signalling activity of Dsh. In contrast, replacing only two basic residues with acidic ones (DshKR/E[2×]) allowed the rescue of the embryonic lethality and of the defects associated with loss of Wnt–Fz//β-catenin signalling, indicating that DshKR/E[2×] can act downstream of Fz to regulate β-catenin. Interestingly, these rescued flies showed PCP defects that correlated with diffuse cytoplasmic localization of DshKR/E[2×]. Membrane localization of DshKR/E[2×] is not, however, fully impaired, because DshKR/E[2×] seemed to be recruited to the plasma membrane on overexpression of Fz. This probably accounts for the ability of DshKR/E[2×] to activate the Wnt–Fz/β-catenin pathway. Two conclusions can be drawn from these results. First, this polybasic cluster is essential for both the localization and activity of Dsh. Second, the Fz/PCP activity of Dsh is more dependent on this DEP–lipid interaction than is its Wnt–Fz/β-catenin activity, suggesting that stable interactions of Dsh with the plasma membrane are required for Fz/PCP signalling, whereas transient interactions may be sufficient for Wnt–Fz/β-catenin signalling (Fig. 1).

How may transient or stable Dsh–lipid interactions translate into distinct signalling outputs? Specificity may arise from thermodynamic differences in the recruitment of Fz/PCP and Wnt–Fz/β-catenin effectors by Dsh. This may be because the localization of Dsh at the plasma membrane during Wnt–Fz/β-catenin signalling may be stabilized through other types of molecular interaction, including its oligomerization[6]. Another possibility is that interaction of the DEP domain with negatively charged lipids might induce a change in the conformation of Dsh that promotes the recruitment of PCP effectors. Other models, involving differential effects of endocytosis for Fz/PCP and Wnt–Fz/β-catenin signalling[7], can also be envisaged.

If signalling output depends on the interaction of Dsh with lipids, then specificity may be linked to the localization of Fz within distinct membrane domains that differ in intracellular

pH and/or lipid composition. It is noteworthy that localization of Fz receptors to the apical membrane of epithelial cells was shown to favour Fz/PCP signalling and basal–lateral localization to promote Wnt–Fz/β-catenin signalling[8]. It will be interesting to examine whether this correlates with differences in lipid composition and/or intracellular pH that may influence Dsh–lipid interaction. Little is known about the spatial and temporal control of local heterogeneities in intracellular pH that may create a microenvironment stabilizing the interaction of Dsh with the plasma membrane. Interestingly, the authors observed that Fz may interact physically with Nhe2, raising the possibility that Fz acts as a feedback control to modulate local intracellular pH. Future studies will certainly test this model further.

Finally, this study provides a very good illustration of the benefit of using cell-based assays and quantitative molecular outputs to obtain mechanistic insights into the regulation of signalling networks[9]. Indeed, although genetic perturbations change the conditions of the initial state of mutant cells, compensatory changes may efficiently buffer these genetic perturbations in living organisms. Thus, direct cell-based readouts can help in identifying key aspects of the cell's physiology that may be more difficult to detect as developmental defects in mutant animals. Nevertheless, validation *in vivo* will remain essential, and genetic tools need to be improved to achieve rapid conditional gene inactivation combined with quantitative phenotypic analysis using molecular readouts.

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Breast cancer quality control

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Tumorigenesis is regulated by several mechanisms including signalling, transcription and DNA replication. Now a cytoplasmic protein quality-control pathway is implicated in the suppression of breast cancer cell growth, suggesting a new role for quality-control mechanisms in suppressing cells with malignant potential.

The tumour biology literature is littered with reports on proteins that inhibit tumour progression. In many cases, the corresponding genes are classified as genuine tumour suppressors because their inactivation leads to spontaneous tumour development. Many of these proteins perform functions logically linked to spontaneous tumour development, such as cell cycle regulation. On page 312 of this issue, Kajiro *et al.* report the surprising observation that expression of the cytoplasmic protein quality-control ubiquitin ligase, CHIP (carboxy terminus of Hsp70-interacting protein) suppresses tumorigenesis and metastatic cellular phenotypes in cultured breast cancer cells[1]. These studies raise the intriguing possibility that quality-control pathways may unexpectedly have a role in tumour progression.

Kajiro *et al.* set out to determine the effects of the overexpression and underexpression of CHIP in mouse tumour xenograft models of breast tumorigenesis, on the basis of their previous demonstration that CHIP can degrade the breast cancer-associated transcription factor oestrogen receptor-α (ER-α) in cell culture systems[2] and their new observation that CHIP mRNA and protein levels vary inversely with the degree of malignancy in human breast tissues[1]. The overexpression of CHIP reduced tumorigenesis of the aggressive metastasis-derived MDA-MB-231 cell line, whereas knockdown of CHIP increased the tumorigenesis of the less aggressive MCF-7 cell line. Surprisingly, these changes did not correlate with the ER-α dependence of tumour cells, and ER-α levels did not vary with changes in CHIP expression. Instead, knocking down CHIP caused the accumulation of the transcriptional coactivator SRC-3. CHIP apparently stimulates the ubiquitylation and degradation of SRC-3. However, other reports indicate that another ubiquitin ligase, Fbw7, is a major determinant of SRC-3 stability in breast and other cell lines[3] and may also function as a tumour suppressor[4]. CHIP-dependent suppression of tumour growth and metastasis can be restored by enforced expression of SRC-3, providing support for Kajiro and colleagues' contention that this is the causative step. Collectively, these studies support a novel mechanism whereby CHIP suppresses tumour growth (Fig. 1).

CHIP has dual activities as a co-chaperone (through its interactions with the cytoplasmic chaperones Hsp70 and Hsp90) and as a quality-control ubiquitin ligase, binding to molecular chaperones and ubiquitylating misfolded proteins to target them for proteasome-dependent degradation[5,6]. Studies *in vivo* have linked CHIP to pathologies associated with protein misfolding, especially in neurological disorders or in response to stress[7,8]. Aberrant protein quality control is broadly associated with chronic degenerative diseases in the nervous system and, more recently, the heart[9]. However, how defects in the protein quality-control machinery would enhance tumorigenesis is less obvious, making Kajiro and colleagues' report quite remarkable in its potential expansion of our understanding of tumour growth. Perhaps aberrant conformations of proteins gain functions that overcome normal cellular checkpoints or otherwise enhance the malignant potential of susceptible cells. Alternatively, CHIP may have functions independent of protein quality-control pathways that affect

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