

Minireview

Dishevelled and Wnt signaling: is the nucleus the final frontier?

Raymond Habas* and Igor B Dawid†

Addresses: *Cancer Institute of New Jersey and Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA. †Laboratory of Molecular Genetics, National Institutes of Child Health and Human Development, Bethesda, MD 20892-2790, USA.

Correspondence: Igor Dawid. E-mail: idawid@nih.gov

Published: 17 February 2005

Journal of Biology 2005, **4**:2

The electronic version of this article is the complete one and can be found online at <http://jbiol.com/content/4/1/2>

© 2005 BioMed Central Ltd

Abstract

The phosphoprotein Dishevelled (Dsh) is an essential component of Wnt signaling pathways and transduces signals into three separate branches, the canonical, non-canonical and Ca²⁺ pathways. How Dsh focuses signaling into these branches remains mysterious, but a new study reveals the importance of nuclear localization of Dsh for pathway-specific activation.

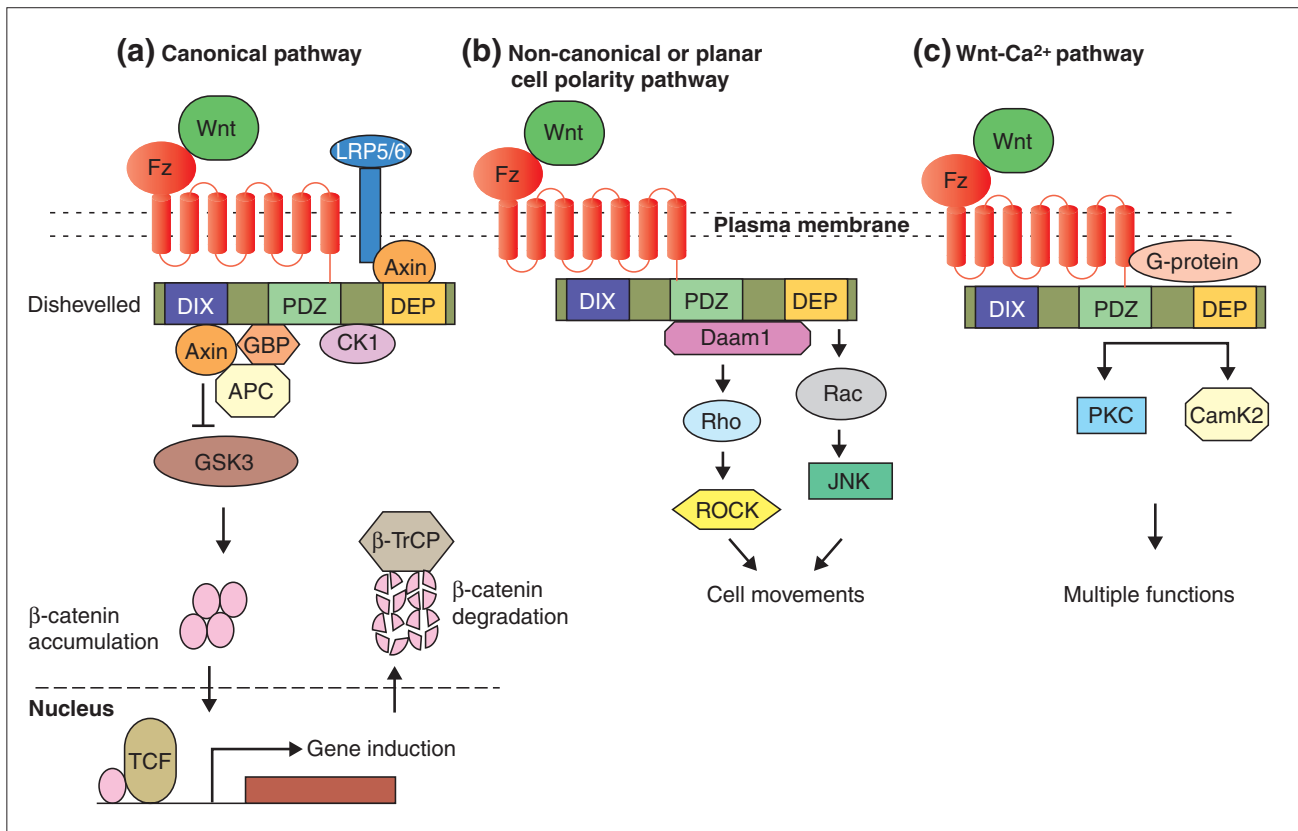
Wnt signaling

Wnt proteins comprise a large family of secreted glycoproteins that regulate key developmental processes including cell-fate determination, proliferation, motility and the establishment of the primary axis of the body during vertebrate embryogenesis [1-3]. Defects in Wnt signaling are also implicated in a host of pathologies including cancer and neural tube defects. Wnt ligands can transform cells, and mutations in components of the Wnt signaling pathway, such as β -catenin, have causative roles in colon cancers in humans, while mutations in Dishevelled (Dsh) are implicated in neural-fold closure disorders [1,4]. To date, 18 Wnt ligands have been identified in humans [5,6]. This large number of ligands is paralleled by an equally impressive number of receptors and co-receptors, which are encoded in the Frizzled and low-density-related lipoprotein receptor 5/6 (LRP5/6) gene families, which have ten and two members, respectively, in the human genome [1,6].

Through intensive studies spanning over two decades, a molecular pathway for Wnt signaling has emerged (Figure 1).

Upon binding of Wnt to its receptor, either Frizzled or a complex comprising Frizzled and LRP5/6, a signal is transduced to the cytoplasmic phosphoprotein Dsh. There are three Dsh proteins in mammals (Dsh-1, Dsh-2, and Dsh-3), and Dsh family members in all organisms are comprised of three highly conserved domains: an amino-terminal DIX domain (named for Dsh and Axin), a central PDZ domain (named for Postsynaptic density-95, Discs-large and Zonula occludens-1), and a carboxy-terminal DEP domain (for Dsh, Egl-10 and Pleckstrin) [7]. At the level of Dsh, the Wnt signal branches into three separate pathways, the so-called canonical, non-canonical or planar cell polarity (PCP), and Wnt-Ca²⁺ pathways (Figure 1) [1,8,9]. In all three pathways Dsh is a key transducer of the Wnt signal that operates at the plasma membrane or in the cytoplasm. But now, a new study [10] suggests that Dsh also functions within the nucleus. To put this study in context, we must first review what is known of the three pathways.

For canonical signaling, which mediates gene induction events (Figure 1a), Wnt signaling utilizes the DIX and PDZ

**Figure 1**

A schematic representation of the Wnt signal transduction cascade. **(a)** For the canonical pathway, signaling through the Frizzled (Fz) and LRP5/6 receptor complex induces the stabilization of β -catenin via the DIX and PDZ domains of Dishevelled (Dsh) and a number of factors including Axin, glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CK1). β -catenin translocates into the nucleus where it complexes with members of the LEF/TCF family of transcription factors to mediate transcriptional induction of target genes. β -catenin is then exported from the nucleus and degraded via the proteosomal machinery. **(b)** For non-canonical or planar cell polarity (PCP) signaling, Wnt signaling is transduced through Frizzled independent of LRP5/6. Utilizing the PDZ and DEP domains of Dsh, this pathway mediates cytoskeletal changes through activation of the small GTPases Rho and Rac. **(c)** For the Wnt- Ca^{2+} pathway, Wnt signaling via Frizzled mediates activation of heterotrimeric G-proteins, which engage Dsh, phospholipase C (PLC; not shown), calcium-calmodulin kinase 2 (CamK2) and protein kinase C (PKC). This pathway also uses the PDZ and DEP domains of Dsh to modulate cell adhesion and motility. Note that for the PCP and Ca^{2+} pathways Dsh is proposed to function at the membrane, whereas for canonical signaling Dsh has been proposed to function in the cytoplasm; a recent study [10] implicates nuclear localization of Dsh in this pathway. See text for further details.

domains of Dsh to induce the stabilization of cytosolic β -catenin; this allows for cytoplasmic accumulation and subsequent translocation of β -catenin into the nucleus [1]. Regulation of β -catenin stability is mediated via a complex of proteins including Axin, glycogen synthase kinase 3 (GSK3), GSK3-binding protein (GBP) and casein kinase 1 (CK1). In the absence of Wnt stimulation, β -catenin is targeted for degradation through the proteosomal pathway via the β -transducin repeat containing protein (β -TrCP), but β -catenin is stabilized when a Wnt signal is received [1,6,11]. In the nucleus, β -catenin forms complexes with members of the LEF/TCF family of transcription factors and other factors, and mediates transcription of Wnt target genes [1].

The non-canonical or PCP pathway mediates cell polarity, cell movements during gastrulation, and other processes, by signal transduction through the PDZ and DEP domains of Dsh, leading to a modification of the actin cytoskeleton (Figure 1b) [8,12]. At the level of Dsh, two independent and parallel pathways lead to the activation of the small GTPases Rho and Rac. Activation of Rho requires the formin-homology protein Daam1 that binds to the PDZ domain of Dsh, leads to the activation of the Rho-associated kinase ROCK, and mediates cytoskeletal re-organization [8,13,14]. Rac activation is independent of Daam1, requires the DEP domain of Dsh, and stimulates Jun kinase (JNK) activity [8,15,16]. Other Dsh-binding molecules that influence the PCP

pathway include Strabismus and Prickle, but their mechanisms of action remain incompletely understood [8,12,17].

The Wnt-Ca²⁺ pathway (Figure 1c) is thought to influence both the canonical and PCP pathways [9]. Wnt signaling through Frizzled receptors leads to the release of intra-cellular Ca²⁺ in a process mediated through heterotrimeric G-proteins and involving numerous other molecules, including phospholipase C (PLC), calcium-calmodulin-dependent kinase 2 (CamK2) and protein kinase C (PKC) [9,18]. The Wnt-Ca²⁺ pathway is important for cell adhesion and cell movements during gastrulation.

Signal specificity

With such a daunting number of Wnt ligands and Frizzled receptors, two challenging questions that remain unanswered are whether (and if so which) Wnt ligands are specific to particular pathways, and how signals are channeled to each pathway. Notably, some Wnt ligands are known to activate both canonical and non-canonical pathways such as Wnt3a, whereas others such as Wnt5a appear to be specific to non-canonical signaling. Equally elusive is the understanding of how the signal is transmitted from the receptor/coreceptor complex to Dsh, although two recent studies have revealed a direct interaction between Dsh and Frizzled [19,20]. Most importantly, the way in which Dsh couples and distributes Wnt signaling into the three signaling branches remains at best poorly understood.

Dsh occupies a key position at the crossroads of all branches of the Wnt signaling cascade. It has been proposed that both the subcellular localization of Dsh and the choice of effector molecules downstream of Dsh govern the selectivity of specific pathway activation. Dsh-localization studies in *Drosophila* [21] and recently *Caenorhabditis elegans* [22] have shown a correlation between localization of Dsh at the membrane and activation of the PCP pathway. Indeed, mutations in the DEP domain, which is required for PCP signaling, show impaired membrane localization that is correlated with impaired PCP signaling [16,21]. These studies have furthered the hypothesis that the membrane localization of Dsh is required for at least one output of Wnt signaling. In unstimulated cells, Dsh localizes to punctate vesicular structures in the cytoplasm [23] by a process that requires the DIX domain; in response to certain Wnt ligands, Dsh translocates to the plasma membrane or to the perinuclear/nuclear area, and the membrane localization in all cases studied requires the DEP domain [24,25]. The significance of nuclear/perinuclear localization remained unclear, but it is noteworthy that a number of components of the canonical signaling pathway, such as APC, Axin, and GSK3 β , appear to traffic between the cytoplasm and the nucleus along with β -catenin [26-28].

This multitude of studies forms the background for the current paper by Sokol and colleagues in *Journal of Biology* [10], which identifies two additional domains in Dsh that modulate both its subcellular distribution and its ability to activate canonical Wnt signaling. The first newly identified domain, located carboxy-terminal to the DEP domain, modulates localization of Dsh through its action as a nuclear export signal. Dsh protein lacking this domain or harboring a mutation in a critical lysine residue strongly accumulates in the nucleus of both *Xenopus* embryos and cultured mammalian cells. Surprisingly, however, these mutant proteins retain their ability to mediate canonical signaling as effectively as wild-type Dsh. Pharmacological agents impeding nuclear export, and cellular fractionation studies, further provide evidence that endogenous Dsh enters the nucleus, supporting the view that Dsh shuttles between the cytoplasmic and nuclear compartments. The authors then identified a second domain, located just carboxy-terminal to the PDZ domain, that is required for nuclear localization; the sequence of this domain is atypical for a nuclear localization sequence (NLS). Mutation of this second domain abolished nuclear accumulation of Dsh in the presence of nuclear export inhibitors and, remarkably, impaired the ability of Dsh to induce β -catenin stabilization and to transduce the canonical Wnt signal. Interestingly, replacement of this atypical NLS with the prototypical NLS of the T antigen from the simian virus SV40 redirected Dsh to the nucleus and largely restored Wnt signaling. The authors further bolster their findings by demonstrating that stimulation of cultured mammalian cells with Wnt3a results in the accumulation of a portion of endogenous Dsh (Dvl2 in this case) in/around the nucleus.

Making sense of nuclear localization

So what is the role for Dsh in the nucleus and is the Wnt field ready to accommodate such a role for Dsh? This new finding [10] comes as a surprise, because Dsh has been studied extensively over the past two decades and its nuclear localization remained unappreciated. To support their conclusions the authors showed that Dsh is found in nuclear fractions, but this approach is not fully conclusive for one may argue that Dsh exhibits perinuclear localization and cofractionates with the outer nuclear envelope. The strongest evidence for a nuclear role for Dsh comes from experiments in which nuclear import and export are manipulated, showing that import is critical for function. Yet, when the basic conclusion of a nuclear localization and function of Dsh is accepted, several questions remain. If Dsh function is required in the nucleus for canonical Wnt signaling, why is no hyperactivation of the pathway observed by targeting Dsh to the nucleus? The authors note this point and postulate that a 'steady state' rather than just localization is

required for function. However, one would at least be compelled to posit that β -catenin, which should be stabilized by such Dsh-targeted approaches, should increase signaling, and this was not observed.

Perhaps a more salient question is why many studies have observed Dsh translocation to the plasma membrane in response to Wnt stimulation or Frizzled expression [8,12,17,29], but have not detected Dsh in the nucleus. It is possible that a small but selective pool of Dsh translocates to the nucleus to mediate canonical signaling while most Dsh goes to the membrane. Yet, if this is the case, is the membrane relocation of the majority of Dsh just a gratuitous cellular behavior without meaning? Finally, what is the function of Dsh in the nucleus? It is possible that nuclear Dsh acts in transcriptional regulation independent of β -catenin to mediate Wnt signaling, as Sokol and colleagues have previously suggested for the Dsh-binding protein Frodo [30]. These remain important questions that no doubt will stimulate future studies. Perhaps the nuclear localization of Dsh will indeed provide clues to elucidate the final frontier of understanding the diverse mechanisms of Wnt regulation of gene transcription in the nucleus.

References

- Logan CY, Nusse R: **The Wnt signaling pathway in development and disease.** *Annu Rev Cell Dev Biol* 2004, **20**:781-810.
- Wodarz A, Nusse R: **Mechanisms of Wnt signaling in development.** *Annu Rev Cell Dev Biol* 1998, **14**:59-88.
- Harland R, Gerhart J: **Formation and function of Spemann's organizer.** *Annu Rev Cell Dev Biol* 1997, **13**:611-667.
- Ueno N, Greene ND: **Planar cell polarity genes and neural tube closure.** *Birth Defects Res C Embryo Today* 2003, **69**:318-324.
- He X: **A Wnt-Wnt situation.** *Dev Cell* 2003, **4**:791-797.
- He X, Semenov M, Tamai K, Zeng X: **LDL receptor-related proteins 5 and 6 in Wnt/ β -catenin signaling: arrows point the way.** *Development* 2004, **131**:1663-1677.
- Wharton KA Jr: **Runnin' with the Dvl: proteins that associate with Dsh/Dvl and their significance to Wnt signal transduction.** *Dev Biol* 2003, **253**:1-17.
- Veeman MT, Axelrod JD, Moon RT: **A second canon. Functions and mechanisms of β -catenin-independent Wnt signaling.** *Dev Cell* 2003, **5**:367-377.
- Miller JR, Hocking AM, Brown JD, Moon RT: **Mechanism and function of signal transduction by the Wnt/ β -catenin and Wnt/Ca²⁺ pathways.** *Oncogene* 1999, **18**:7860-7872.
- Itoh I, Brott BK, Bae GU, Ratcliffe MJ, Sokol S: **Nuclear localization is required for Dishevelled function in Wnt/ β -catenin signalling.** *J Biol* 2005, **4**:3.
- Liu C, Kato Y, Zhang Z, Do VM, Yankner BA, He X: **β -Trcp couples β -catenin phosphorylation-degradation and regulates *Xenopus* axis formation.** *Proc Natl Acad Sci USA* 1999, **96**:6273-6278.
- Wallingford JB, Fraser SE, Harland RM: **Convergent extension: the molecular control of polarized cell movement during embryonic development.** *Dev Cell* 2002, **2**:695-706.
- Habas R, Kato Y, He X: **Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1.** *Cell* 2001, **107**:843-854.
- Marlow F, Topczewski J, Sepich D, Solnica-Krezel L: **Zebrafish Rho kinase 2 acts downstream of Wnt11 to mediate cell polarity and effective convergence and extension movements.** *Curr Biol* 2002, **12**:876-884.
- Habas R, Dawid IB, He X: **Coactivation of Rac and Rho by Wnt/Frizzled signaling is required for vertebrate gastrulation.** *Genes Dev* 2003, **17**:295-309.
- Boutros M, Paricio N, Strutt DJ, Mlodzik M: **Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling.** *Cell* 1998, **94**:109-118.
- Keller R: **Shaping the vertebrate body plan by polarized embryonic cell movements.** *Science* 2002, **298**:1950-1954.
- Kuhl M: **Non-canonical Wnt signaling in *Xenopus*: regulation of axis formation and gastrulation.** *Semin Cell Dev Biol* 2002, **13**:243-249.
- Wong HC, Bourdelas A, Krauss A, Lee HJ, Shao Y, Wu D, Mlodzik M, Shi DL, Zheng J: **Direct binding of the PDZ domain of Dishevelled to a conserved internal sequence in the C-terminal region of Frizzled.** *Mol Cell* 2003, **12**:1251-1260.
- Cong F, Schweizer L, Varmus H: **Wnt signals across the plasma membrane to activate the β -catenin pathway by forming oligomers containing its receptors, Frizzled and LRP.** *Development* 2004, **131**:5103-5115.
- Axelrod JD, Miller JR, Shulman JM, Moon RT, Perrimon N: **Differential recruitment of Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways.** *Genes Dev* 1998, **12**:2610-2622.
- Walston T, Tuskey C, Edgar L, Hawkins N, Ellis G, Bowerman B, Wood W, Hardin J: **Multiple Wnt signaling pathways converge to orient the mitotic spindle in early *C. elegans* embryos.** *Dev Cell* 2004, **7**:831-841.
- Capelluto DG, Kutateladze TG, Habas R, Finkielstein CV, He X, Overduin M: **The DIX domain targets Dishevelled to actin stress fibres and vesicular membranes.** *Nature* 2002, **419**:726-729.
- Torres MA, Nelson WJ: **Colocalization and redistribution of dishevelled and actin during Wnt-induced mesenchymal morphogenesis.** *J Cell Biol* 2000, **149**:1433-1442.
- Endo Y, Wolf V, Muraiso K, Kamijo K, Soon L, Uren A, Barshishat-Kupper M, Rubin JS: **Wnt-3a-dependent cell motility involves RhoA activation and is specifically regulated by Dishevelled-2.** *J Biol Chem* 2004, **280**:777-786.
- Franca-Koh J, Yeo M, Fraser E, Young N, Dale TC: **The regulation of glycogen synthase kinase-3 nuclear export by Frat/GBP.** *J Biol Chem* 2002, **277**:43844-43848.
- Wiechens N, Heinle K, Englmeier L, Schohl A, Fagotto F: **Nucleo-cytoplasmic shuttling of Axin, a negative regulator of the Wnt- β -catenin pathway.** *J Biol Chem* 2004, **279**:5263-5267.
- Cong F, Varmus H: **Nuclear-cytoplasmic shuttling of Axin regulates subcellular localization of β -catenin.** *Proc Natl Acad Sci USA* 2004, **101**:2882-2887.
- Rothbacher U, Laurent MN, Deardorff MA, Klein PS, Cho KW, Fraser SE: **Dishevelled phosphorylation, subcellular localization and multimerization regulate its role in early embryogenesis.** *EMBO J* 2000, **19**:1010-1022.
- Hikasa H, Sokol SY: **The involvement of Frodo in TCF-dependent signaling and neural tissue development.** *Development* 2004, **131**:4725-4734.