A classical way to investigate the functions of a protein is to start by defining where it is distributed. Membrane-spanning proteins often function as receptors involved in recognition and cell adhesion, whereas nuclear proteins frequently play a role in regulating gene expression and transcription. But it is becoming increasingly clear that protein subcellular localization can be extremely dynamic, allowing key proteins to play different roles in different compartments. Now, in *Journal of Biology* [1], Sergei Sokol and colleagues show that the Dishevelled (Dsh) protein of the Wnt signaling pathway can shuttle in and out of the nucleus (see ‘The bottom line’ box for a summary of the work and ‘Background’ for further explanations and definitions). These observations challenge the conventional thinking about Dsh function and suggest that Dsh might do very different things depending on where it is in the cell.

**Canonical and non-canonical Wnt pathways**

During growth, development and disease, extracellular signals are communicated, or transduced, into the cell and in such a way as to elicit a particular cellular response. Many key signal transduction pathways have been dissected using genetic and biochemical approaches; such studies have defined the molecules that ensure signals initiated at the cell surface are efficiently transmitted to the cell nucleus, where they often result in the induction of a specific gene-expression program. Many signal transduction pathways are composed of modules that are remarkably conserved across species, such that lessons from different experimental model organisms have contributed to the understanding of molecular hierarchies that control signal communication in many cellular contexts.

Studies of the Wnt pathway provide a wonderful example of how researchers from different fields have contributed to a detailed understanding of a key signal transduction pathway [2]. The Wnt pathway is critical for development and homeostasis of animals from *Hydra* to human [3]; Wnt signaling regulates cell proliferation, cell polarity and cell-fate determination. The Wnt signaling machinery is tightly regulated, and disruption of components of the signaling pathway have been implicated in diseases including cancer [2,4].
Background

- The **Dishevelled (Dsh)** gene was first identified in *Drosophila* by the description of a mutant with defects in the arrangement of bristles on the wing and thorax. Dsh mutant phenotypes are reminiscent of wingless and armadillo mutant embryos; Wingless is the fly equivalent of mammalian Wnt proteins and armadillo encodes the fly β-catenin protein.

- Secreted Wnt proteins associate with members of the Frizzled family of seven transmembrane-domain receptors on the cell surface. Wnt binding induces the phosphorylation of Dsh protein; Dsh can block the activity of Axin, a cellular inhibitor of the Wnt signaling pathway (see Figure 1).

- Wnt signaling is implicated in many biological processes including cell proliferation, cell polarity and cell-fate specification. The ‘canonical’ Wnt/β-catenin signaling pathway links Wnt signaling to stabilization of the β-catenin protein; β-catenin in turn translocates to the nucleus and interacts with T cell-specific transcription factor (TCF) to drive the expression of target genes. Wnt signaling can also lead to activation of at least one non-canonical pathway involved in regulating planar cell polarity.

- Dsh is composed of three conserved domains: the amino-terminal DIX domain, which is found in Dsh and Axin; the central PDZ domain (found in Postsynaptic density-95, Discs-large and Zonula occludens-1 proteins); and the DEP domain (found in Dsh, Egl-10 and pleckstrin).

- The dynamics of protein localization inside the cell can be influenced by the activity of nuclear localization signals (NLS) and nuclear export signals (NES) that regulate protein shuttling into and out of the nucleus, respectively.

The first step in the Wnt signal occurs when extracellular Wnt ligand binds Frizzled receptors on the cell surface, leading to the activation of several distinct transduction pathways (see Figure 1). The canonical Wnt pathway involves stabilization of the intracellular protein β-catenin. The degradation of β-catenin is regulated by interaction with a number of proteins including Axin, glycogen synthase kinase 3 (GSK3) and the adenomatous polyposis coli protein (APC). The degradation machinery is inhibited by Dsh, leading to the accumulation of β-catenin, which in turn translocates to the nucleus and initiates a gene expression program by interacting with transcription factors such as T-cell-specific transcription factor (TCF). Frizzled receptors can also initiate an independent non-canonical Wnt pathway that diverges to regulate complex developmental events involved in planar cell polarity and convergent extension movements during embryo development, via small GTPases and the JNK kinase. The intracellular protein Dishevelled is common to both canonical and non-canonical signaling pathways, raising the question of how this mysterious protein acts at the signal crossroads.

**Dishevelled distribution**

The Dishevelled protein was first discovered in flies, and several homologs have been found in other organisms including mammals [3]. Analysis of Dsh sequence alignments revealed the presence of three conserved domains called DIX, PDZ and DEP [5,6], which are implicated in protein-protein interactions and targeting to subcellular sites. The modular design of the Dsh protein suggested that different domains might function to route Wnt-Frizzled signals in different directions.

Sokol’s group at Harvard Medical School decided to test this hypothesis by making mutant forms of Dsh that lack different domains and fusing them to green fluorescent protein (GFP) to track their subcellular localization (see the ‘Behind the scenes’ box for more of the rationale for the work). When they expressed the full-length Dsh-GFP protein in *Xenopus* ectoderm cells they observed spotty staining in the cytoplasm, but a Dsh protein lacking the DEP domain appeared in the nucleus. Initially perplexed, Sokol and colleagues then scanned the Dsh polypeptide for a leucine-rich nuclear export sequence (NES). They found one and mutated it to demonstrate that normally Dsh is efficiently exported from the nucleus by means of this sequence. The NES mutant accumulated in the nucleus, but it could still function normally, inducing a secondary dorsal axis when injected into frog embryos (a classical developmental assay for Dsh biological activity). The team confirmed the nuclear shuttling of Dsh using drugs that block active nuclear export; these drugs led to nuclear accumulation of endogenous Dsh proteins in mammalian cells.

“Once we had seen nuclear export we wanted to find out if there was an import signal,” recalls Sokol. His team hunted for a conventional nuclear localization signal (NLS). “This was probably the most difficult part of the study, because the Dsh NLS doesn’t match the known consensus.” The team began a mutagenesis program, hacking away at the protein until they narrowed the NLS down to a short stretch of residues between the PDZ and DEP domains. “The Dsh NLS is
atypical; it doesn’t look like anything else,” comments Sokol. “But it’s very conserved between Dsh proteins across species, so it may be a specialized way for Dsh to get into the nucleus.” Removing the NLS blocked nuclear import. NLS-mutant proteins also failed to induce secondary axes in frog embryos, or to stabilize β-catenin and activate downstream target genes. When the Dsh NLS was replaced with an unrelated NLS from a viral protein, Dsh activity was restored, as was canonical Wnt signaling. In contrast, the Dsh NLS mutation did not affect non-canonical Wnt signaling. Finally, Sokol’s group showed that endogenous Dsh relocates to the nucleus in mammalian cells upon Wnt stimulation.

**Dishevelled’s nuclear shuffle**

Sokol notes that some early reports mentioned nuclear localization of Dsh [7,8], but these did not address the functional importance of Dsh nuclear accumulation in Wnt signaling. Randall Moon’s group at the University of Washington in Seattle had noticed Dsh in the nucleus in association with the Dapper protein [8]. “What is interesting is the Sokol finding that blocking nuclear export leads to accumulation in the nucleus, suggesting that Dsh nuclear accumulation is regulated,” says Moon. “This is a careful study which provides compelling evidence for Dsh nuclear import,” adds Howard Hughes Investigator Norbert Perrimon, who (independent from Sokol) works at Harvard Medical School.

“These results bring a new level of complexity to the regulation of the Wnt signaling pathway,” agrees Patricia Salinas from University College London, UK. Her group previously showed that Dsh binds to microtubules and locally regulates signaling events in neuronal axons [9]. She notes that a large number of Wnt signaling components have recently been found in the nucleus. “These results fit very well with our view that Dsh regulates distinct signaling events in specific cellular compartments. Our task now is to elucidate how the localization of Dishevelled is regulated. For example, what determines its re-localization to the nucleus or to microtubules?” Perrimon notes that “the issue of Dsh nuclear localization needs to be re-examined in the other systems to find out how general this is.”

Sokol’s results have met with some resistance from the Wnt community. Moon notes that often it takes years to change peoples’ ideas about Wnt signaling. He cites the example of Frizzled receptors signaling via heterotrimeric G proteins, which he proposed years ago and which has only recently been clearly demonstrated. Sokol points out that β-catenin itself was originally described in cell adhesion. “People didn’t believe that it goes to the nucleus until much later.” Sokol is sure that many of the Wnt signaling proteins have nuclear functions.

“What remains completely opaque is what Dsh is doing in the nucleus and with whom,” says Moon. Dsh has been reported to interact with over a dozen proteins, including several kinases [6]. Sokol speculative that Dsh may have nuclear rooles beyond the stabilization of β-catenin. For example, he notes that the recently identified Frodo protein binds to Dsh and Tcf proteins independent of β-catenin and may serve as a bridge to regulate gene expression [10]. “I would say we are just at the tip of the iceberg,” says Sokol. “There may be huge Dsh nuclear complexes that control chromatin structure or assembly.” All in the field appear to agree that cellular localization offers possibilities for distinct functions in different compartments. “It remains a question whether Dsh mobilization to the

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**Figure 1**

Wnt signals via canonical and non-canonical pathways. Both start when Wnt ligand binds the Frizzled receptor at the cell surface, and both include the key mediator Dsh. Dsh is made up of three major motifs plus nuclear import (NLS) and export signals (NES); the red arrow indicates the newly identified direct route Dsh takes into the nucleus. The roles of the other proteins in the two pathways, and of the Dsh motifs, are discussed in the text.
nucleus depends on the cellular context or the amount of Wnt that the cell encounters,” adds Salinas. “We truly understand only a fraction of the molecules involved in Wnt signaling,” admits Moon. New technologies are revealing many new components of the Wnt β-catenin pathway. Several of these are nuclear and could interact with Dsh. “Thus many surprises await us,” predicts Moon. “It’s a pleasure to learn that we still have much that we barely understand.”

References
1. Itoh K, Brott BK, Bae GU, Ratcliffe MJ, Sokol SY: Nuclear localization is required for Dishevelled function in Wnt/β-catenin signaling. J Biol 2003, 4:3.
2. Peifer M, Polakis P: Wnt signaling in oncogenesis and embryogenesis - a look outside the nucleus. Science 2000, 287:1606-1609.
3. The Wnt Homepage [http://www.stanford.edu/~rnusse/wntwindow.html]
4. Bienz M, Clevers H: Linking colorectal cancer to Wnt signaling. Cell 2000, 103:311-320.
5. Boutros M, Mlodzik M: Dishevelled: at the crossroads of divergent intracellular signaling pathways. Mech Dev 1999, 83:27-37.
6. Wharton KA: Runnin’ with the Dvl: proteins that associate with Dsh/Dvl and their significance to Wnt signal transduction. Dev Biol 2003, 253:1-17.
7. Torres MA, Nelson WJ: Colocalization and redistribution of Dishevelled and Actin during Wnt-induced mesenchymal morphogenesis. J Cell Biol 2000, 149:1433-1442.
8. Cheyette BNR, Waxman JS, Miller JR, Takeharu KI, Sheldahl LC, Khlebtsova N, Fox EP, Earnest T, Moon RT: Dapper, a Dishevelled-associated antagonist of β-catenin and JNK signaling, is required for notochord formation. Dev Cell 2002, 2:449-461.
9. Ciani L, Krylova O, Smalley MJ, Dale TC, Salinas PC: A divergent canonical WNT-signaling pathway regulates microtubule dynamics: dishevelled signals locally to stabilize microtubules. J Cell Biol 2004, 164:243-253.
10. Hikasa H, Sokol SY: The involvement of Frodo in TCF-dependent signaling and neural tissue development. Development 2004, 131:4725-4734.

Jonathan B Weitzman is a scientist and science writer based in Paris, France.
E-mail: jonathanweitzman@hotmail.com