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Dishevelled nuclear shuttling

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Structure-function analysis of the Dishevelled (Dsh) protein in frog embryos has defined sequences that regulate Dsh nuclear localization, which proves critical for Wnt signaling.

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].

The bottom line

- Sokol and colleagues found that the Dishevelled (Dsh) protein of the Wnt signaling pathway is found in the cell nucleus in response to Wnt signaling.
- Mutation of a nuclear export signal in Dsh leads to accumulation of Dsh in the nucleus but does not impair canonical Wnt signaling.
- Mutation of the nuclear localization signal (NLS) in Dsh disrupts the protein's ability to activate downstream target genes; this activity can be restored by replacement of the relevant sequence with an unrelated NLS.
- Nuclear localization of Dsh appears to be essential for its function in the canonical Wnt-β-catenin signaling pathway.

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

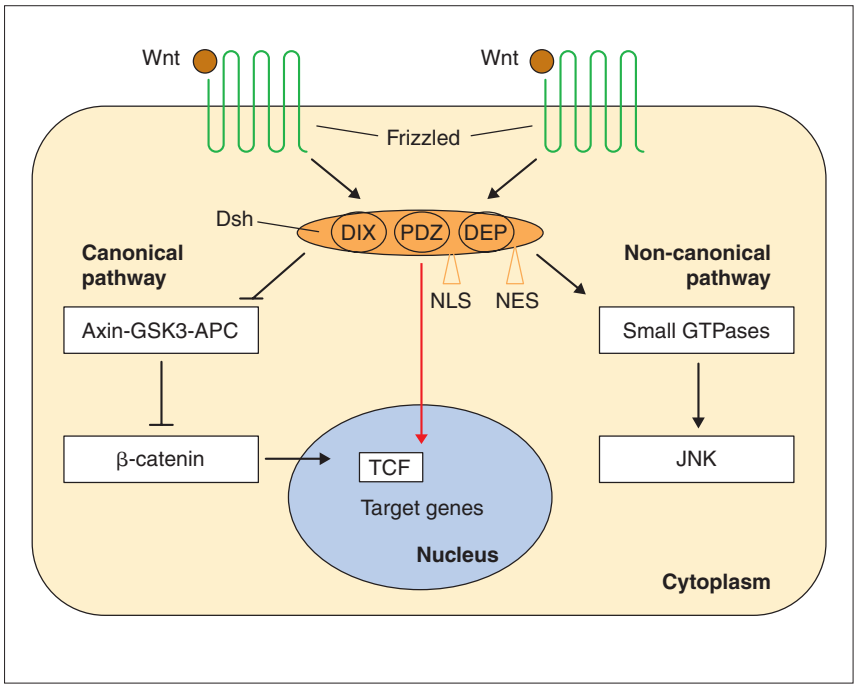

Figure 1
Wnt signals via canonical and non-canonical pathways. Both start when Wnt ligand binds the Frizzled receptor at the cell surface, and both include the key mediator Dsh. Dsh is made up of three major motifs plus nuclear import (NLS) and export signals (NES); the red arrow indicates the newly 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.

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 speculates that Dsh may have nuclear roles 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

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."

Behind the scenes

Journal of Biology asked Sergei Sokol about his studies of Dishevelled localization and function.

What prompted you to study Dishevelled localization?

Dishevelled (Dsh) is known to be involved in different signaling pathways and one explanation might be that different signaling events take place in different cell compartments. When we started to break the Dsh protein into domains and to analyze the properties of the individual domains, we found that the Dsh mutant missing the DEP domain ended up in the nucleus and at the same time it was fully active in terms of canonical signaling to Wnt target genes. So, we figured that this might be critical to understanding Dsh regulation.

How long did it take to do the experiments and what were the steps that ensured success?

We obtained the key result, the accumulation of Dsh protein in the nucleus upon treatment with a nuclear export inhibitor, quite early on. This assured us that the project was viable, and most of the other experiments were done in a few months. We had to establish a reliable cell fractionation technique to separate nuclei from cytoplasm and to obtain antibodies that specifically recognize endogenous Dsh. Another critical step was to show that Dsh can translocate to the nucleus in response to Wnt ligands. The use of *Xenopus* embryos allowed us to assess the functional activity of different Dsh mutants in reproducible assays that take only a few weeks.

What was your initial reaction to the results and how were they received by others?

We were quite surprised initially and set out to show that nuclear Dsh is functional. Our results don't fit the general consensus view in which β-catenin is the major factor that controls activation of Wnt target genes in the nucleus. We suggest that Dsh in the nucleus may function in a new branch of the Wnt pathway that is independent of β-catenin stabilization. Many Wnt researchers were also surprised, as this raised questions about what Dsh might be doing in the nucleus.

What are the next steps?

We would still like to know how Dsh functions and what it is doing in the nucleus. We are investigating the function of proteins that bind to Dsh at different locations, so that we can separate different branches of Dsh control pathways. We would like to explore the possibility that Dsh regulates chromatin structure and associates with chromatin or nuclear complexes.

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