Journal Club

A Wingless Flight

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The Wnt/Wingless (Wg) pathway is an evolutionarily conserved signaling pathway that plays important roles in normal development and cancer, from worms and flies to mice and humans. Two papers, identifying the first components of this pathway, initiated the active research field of Wnt/Wg signal transduction and profoundly influenced my scientific career.

In 1994 two groups, led by Norbert Perrimon and Roel Nusse, identified the first components of the Wg signaling pathway (Noordermeer et al. 1994; Siegfried et al. 1994) in the fruitfly *Drosophila melanogaster*. Embryos lacking the segment polarity gene *wingless* (*wg*) are completely covered with small pointed ridges (denticles) on their undersurface (ventral cuticle),

Journal Club is a forum for postdoctoral scientists and graduate students to discuss an important paper in the context of their own scientific interests.

Abbreviations: APC, adenomatous polyposis coli; *arm*, *armadillo*; *dsh*, *dishevelled*; GSK3, glycogen synthase kinase 3; LEF/TCF, lymphoid enhance factor/T cell factor; *porc*, *porcupine*; *Ubx*, *Ultrabithorax*; *wg*, *wingless*; WRS, Wingless response sequence; *zw3*, *zeste-white 3*

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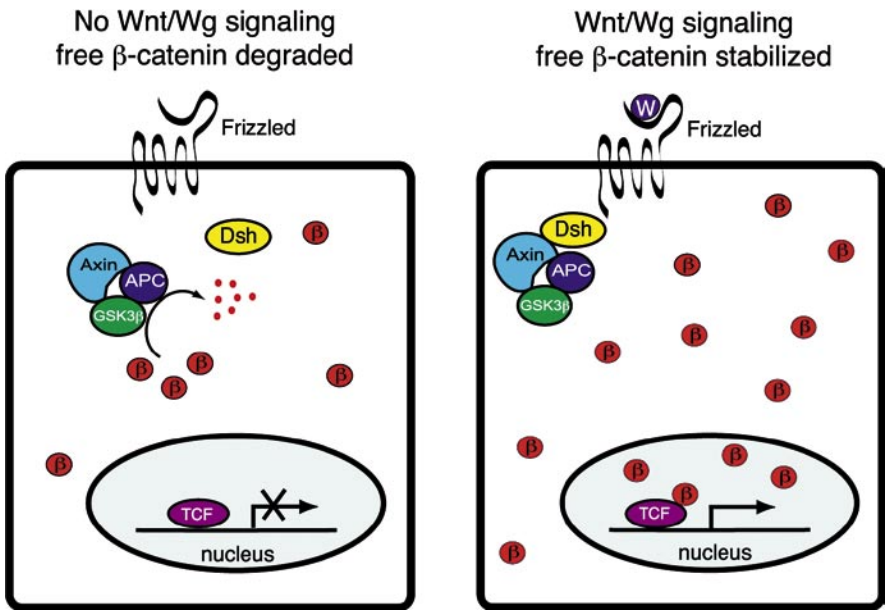
DOI: 10.1371/journal/pbio.0000049

in contrast to wild-type embryos, which have an alternating pattern of naked cuticle and denticle belts (Cadigan and Nusse 1997). Similar phenotypes were observed in a number of other mutations, including *dishevelled* (*dsh*), *porcupine* (*porc*), and *armadillo* (*arm*), while mutation in *zeste-white 3* (*zw3*) or overexpression of Wg resulted in the opposite phenotype, i.e., an entirely naked cuticle. To investigate the relationship among these genes, the two groups exploited genetic epistasis, a manipulation in which two mutations with different phenotypes are combined in a single organism: if the genes are components of the same signaling pathway, the double mutant would resemble mutation in the more downstream component. By combining pairs of mutations with opposite phenotypes and observing the resulting effect, the investigators ordered the genes in a single signaling pathway: *porc* is required for secretion of Wg, while *zw3* acts downstream of *dsh* and upstream of *arm* to transduce the Wg signal in the recipient cell. Thus, the first four components of the Wnt signaling pathway were identified (see Figure 1).



DOI: 10.1371/journal/pbio.0000049.g001

Figure 1. A Simplified Version of Wnt/Wg Signaling
In the absence of the Wnt ligand, Arm/β-catenin (β) is phosphorylated by a complex of Axin, APC, and Zw3/GSK3β and rapidly degraded. Upon Wnt (W) signaling through the Frizzled receptor and Dsh, this complex is inhibited: as a consequence, β-catenin accumulates and binds to LEF/TCF proteins to stimulate transcription of Wnt target genes.

When these papers came out in 1994, I was a second-year undergraduate looking for some research experience over the summer in the laboratory of Mariann Bienz at the Laboratory of Molecular Biology in Cambridge, United Kingdom. At the time, Mariann's group was studying transcriptional regulation of the homeobox gene *Ultrabithorax* (*Ubx*) in the developing *Drosophila* midgut and had already discovered that *Ubx* expression was regulated by Wg through a so-called Wg response sequence (WRS). Publication of these papers by the Perrimon and the Nusse labs immediately prompted the question of whether the newly identified Wg pathway components transduced the Wg signal in the *Drosophila* midgut in addition to the epidermis. And, if so, did these components act through the WRS of the *Ubx* promoter? My summer project thus began at the cutting edge of research on a novel signaling pathway. I was to repeat the epistasis experiments using the *dsh*, *zw3*, and *arm* mutants and observe the effects of these mutations in the midgut, especially on reporters containing the WRS.

With a lot of beginner's luck, I found that *dsh*, *zw3*, and *arm* transduce the Wg signal in the midgut, just as in the epidermis, and that they act through the 12 bases in the *Ubx* promoter that Mariann's group identified as the WRS. Everyone in the lab was excited because the Wg pathway seemed to be conserved, and we were one step closer to identifying the mysterious transcription factor responsible for mediating the Wg signal. I was thrilled because, by the end of the summer, I had most of the results required for a first-author publication (Yu et al. 1996) and a full scholarship to come back and work with Mariann as a graduate student.

At that time, among the components of the Wg pathway, *porc* was uncharacterized, *dsh* encoded a novel intracellular protein of unknown function, Zw3 was a serine/threonine kinase homologous to glycogen synthase kinase 3 (GSK3), and Arm was the homologue of β-catenin, a cell adhesion molecule. There were no known interactions between any of these components; the Wg receptor and nuclear target were lacking, and the most downstream protein identified was a cell adhesion molecule with no known role in transcriptional regulation. However, since Arm was the most downstream component of the Wg pathway known and Mariann's group was interested in identifying the nuclear target of Wg signaling, Arm was the obvious candidate to start with. I was using a yeast two-hybrid screen to look for proteins that interact with Arm, when through the grapevine came the news that the lymphoid enhance factor/T cell factor (LEF/TCF) binds to β-catenin, the mammalian homologue of Arm. So we did experiments to show that mouse Lef-1 also binds to Arm and that together they activate transcription through the WRS in the *Ubx* promoter (Riese et al. 1997). The identification of the LEF/TCF proteins as the nuclear target of Wnt signaling is a beautiful example of how research in different organisms and the use of different approaches converge to tell a complete story. The LEF/TCF proteins were first

cloned as enhancer-binding factors in T cells, but they were poor transcriptional activators until their interaction with β-catenin was identified. Together, LEF/TCF and β-catenin function as potent transcriptional activators. The overexpression work identifying LEF/TCF proteins as components of the Wnt pathway were mostly done in *Xenopus* embryos (Behrens et al. 1996; Huber et al. 1996; Molenaar et al. 1996), while the loss-of-function mutation (Brunner et al. 1997; van de Wetering et al. 1997) and the effects of LEF-1 on an identified WRS (Riese et al. 1997) were done in *Drosophila*. It has thus become clear at this point that the Wnt/Wg signaling pathway is highly conserved in evolution.

Ten years after the initial characterization of the Wnt/Wg signaling pathway, nearly 50 components have been identified (Cadigan and Nusse 1997; Bienz and Clevers 2000; Polakis 2000; see also http://www.stanford.edu/~rnusse/wntwindow.html). In addition to receptors and nuclear targets, proteins have been identified that regulate the activity of this pathway at the level of ligand binding, β-catenin stability, and transcription. Mutations that allow β-catenin to accumulate in the absence of Wnt signaling, such as truncations in the adenomatous polyposis coli (APC) protein and stabilizing mutations in β-catenin, can lead to cancer. In the yeast two-hybrid screen that I started at the beginning of graduate school, I identified a highly expressed *Drosophila* homologue of APC and showed that the localization of Arm and APC to the adhesive zones of epithelial cells is very important for their function (Yu et al. 1999).

I still look to these first Wg pathway papers as my real introduction to hands-on scientific research. They opened a new field that I have been working in ever since. Following graduate research with Mariann, I moved up the evolutionary ladder to work on cultured hippocampal neurons from rat brains in the laboratory of Robert Malenka at Stanford University, bringing my deep interest in β-catenin and Wnt signaling to a new system. During my postdoctoral work, I have identified β-catenin, in its cell adhesion function as part of the cadherin/catenin complex, as an important mediator of dendritic morphogenesis (Yu and Malenka 2003). I have also shown that Wnt signaling plays a role in dendritic development in a transcriptionally independent manner. It is very exciting to have identified a new function for my favorite molecule in a model system for which relatively little is known about the function of Wnt/β-catenin signaling. I hope to be able to start my own research group in the near future to work on the role of Wnt/β-catenin signaling in dendritic development and neural circuit formation. ■

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