Please cite this article in press as: van Amerongen, Celebrating Discoveries in Wnt Signaling: How One Man Gave Wings to an Entire Field, Cell (2020), https://doi.org/10.1016/j.cell.2020.03.033

Celebrating Discoveries in Wnt Signaling: How One Man Gave Wings to an Entire Field

Renée van Amerongen[1,2,*]
[1]Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, the Netherlands
[2]Twitter: @wntlab
*Correspondence: r.vanamerongen@uva.nl
https://doi.org/10.1016/j.cell.2020.03.033

This year's Gairdner Foundation Award for Biomedical Research goes to Roel Nusse for his pioneering work on the Wnt signaling pathway and its many roles in development, cancer, and stem cells.

All multicellular animals face the daunting task of properly organizing their cells into complex, specialized tissues. They do not only have to build these structures during embryonic development but also actively maintain them to preserve homeostasis later in life. A tissue, therefore, is a dynamic entity in which cells actively turn over. The inability to balance cell proliferation and differentiation in this context is a recipe for disaster, resulting in either unbridled cell division (risking tumor formation) or loss of tissue integrity (contributing to degenerative diseases and aging).

In the past 40 years, the Wnt signal transduction pathway has emerged as a cell-to-cell communication pathway with an important role in each of the aforementioned processes. Conserved in all multicellular animals, it is one of the oldest developmental signaling pathways, controlling robust pattern formation in the early embryo in myriad species. Interest in Wnt signaling stretches far beyond the developmental and evolutionary biology communities, however, and research in the field has impacted on many areas of biomedical research. None of this would have been possible without the contributions of Roel Nusse, who has, in many ways, shaped the field itself from the very start (Figure 1).

Unbeknownst to him at the time, Nusse stumbled upon the Wnt pathway in the late 1970s, when he was a PhD student in what was then the Division of Virology at the Netherlands Cancer Institute in Amsterdam. His research focused on the mouse mammary tumor virus (MMTV), a retrovirus that had long been known to cause mammary tumor formation in certain inbred mouse strains. The mechanism, however, remained entirely unknown. His was another project in a long tradition of MMTV studies that had been ongoing at the Netherlands Cancer Institute since the 1930s.

Let us pause for a moment to sketch the biomedical research landscape at the time. In 1975, the potential hazards of recombinant DNA technology (i.e., the possibility to cut and paste together pieces of DNA from different species for the first time in history, something referred to as "DNA cloning" and nowadays one of the most basic techniques in any molecular biology lab) had been discussed at the famous Asilomar conference. This had resulted in a set of guidelines under which experiments using the technology were judged safe to continue. Up until then, it had been virtually impossible for researchers to get their hands on specific DNA sequences—let alone use them for experimentation. Around the same time, the question of what caused cancer was also a hot topic of debate. Cumulative research had just revealed that the fast-transforming Rous sarcomavirus carried a transforming oncogene, later to be known as *v-src*. Other, slow-transforming viruses, including MMTV, seemed devoid of such a load. Then came the Nobel prize-winning work of Harold Varmus and Peter Bishop, which showed that *v-src* had a non-viral origin: somehow, the virus had at one point hijacked a cellular gene (*c-src*). This so-called proto-oncogene was found to be present in the genome of many different species, suggesting that cancer too might have a cellular origin and that, perhaps, slow-transforming retroviruses could induce cancer by activating these proto-oncogenes.

Roel Nusse initially studied the infection mechanism of MMTV as well as the characteristics of some of its proteins. But in 1980, researchers at the institute had gotten their hands on recombinant DNA technology, and the expectation was that this would help reveal what changes were brought about by integration of MMTV into mammary epithelial cells. By then, Nusse had also come into contact with Harold Varmus, who was equally intrigued by the question of how MMTV caused mammary tumors to form. Nusse joined Varmus for a postdoc at the University of California in San Francisco. The offer letter (archived by the US National Library of Medicine) sketches a long list of interesting projects, mentioning what would later become groundbreaking work as almost an afterthought in the P.S.: "I should have mentioned the possibility of looking directly at the 'dominant' proviruses in tumor DNA." Together, Nusse and Varmus indeed set out to find a tumor with a clonal MMTV insertion in which they could ultimately map the precise proviral integration site: in this particular locus (*int-1*), the virus had landed in the vicinity of an unknown gene, inducing its enhanced expression via the strong enhancer sequences in the viral LTRs. Many other, independent tumors also harbored a proviral integration in *int-1*, suggesting a causal role for this cellular gene product in mammary tumor formation. One year later, the structure of the gene had been mapped. Barring any homology to known oncogenes, this represented the discovery of a novel cellular

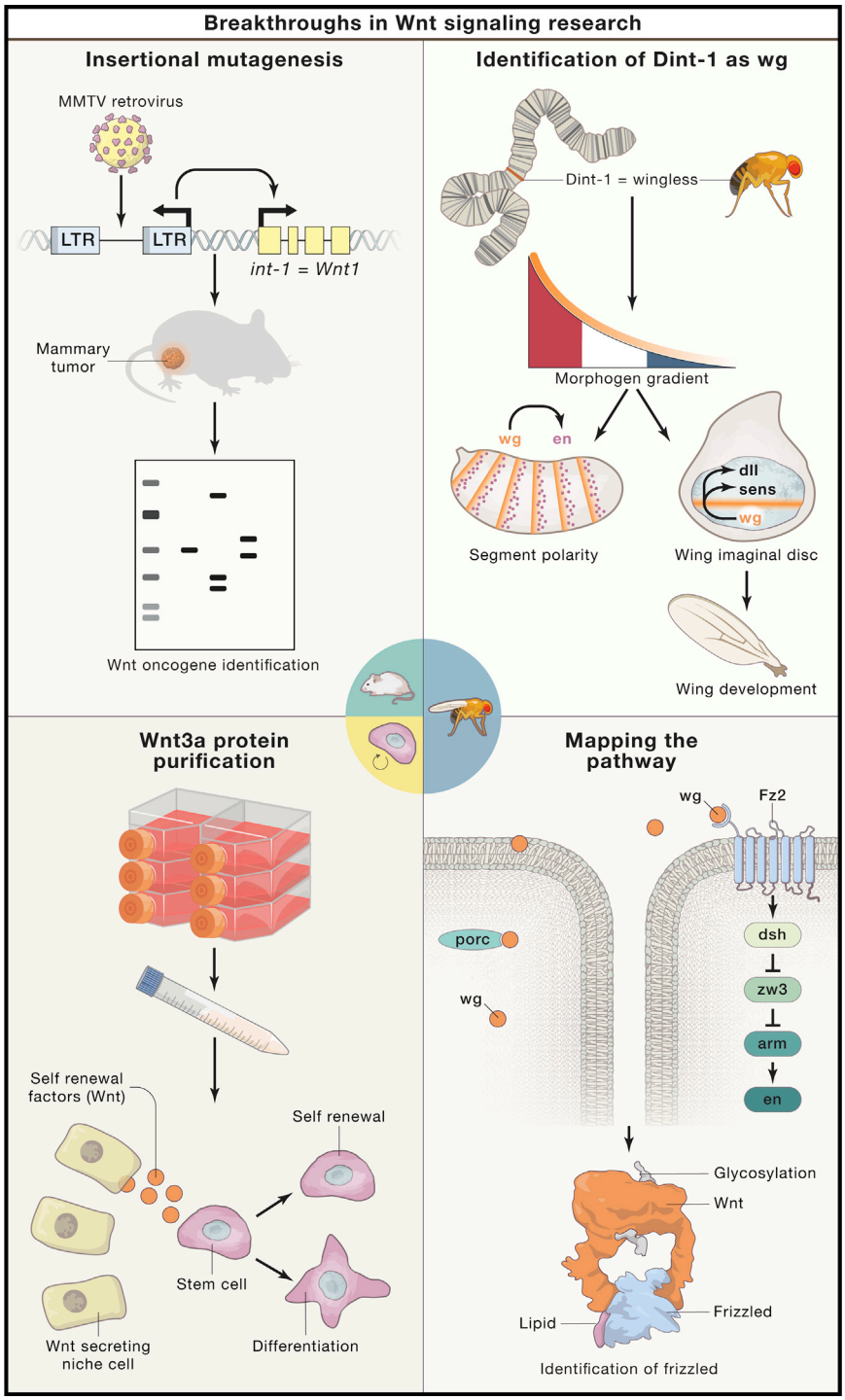

Figure 1. Breakthroughs in Wnt Signaling Research

(Top left) The principle behind insertional mutagenesis as a method for oncogene identification using slow-transforming retroviruses such as MMTV. Proviral integrations occur randomly throughout the genome. If a proviral insertion in the vicinity of a given gene yields a selective advantage, for instance by resulting in overexpression of a growth-promoting gene, it can be identified as a prominent or clonal mutation in the resulting tumor. By searching for so-called common integration sites (i.e., integrations of the provirus in the same genetic locus across multiple independent tumors), new cellular oncogenes can be identified.

(Top right) Identification of the *wingless* mutant as the *Drosophila int-1* homolog (*Dint-1*) using classical mapping on polytene chromosomes. The *wingless* protein (*wg*) is a classical morphogen that forms gradients in a tissue. Cells close to the site of *wg* production encounter high levels of the protein (resulting in the induction of high-threshold target genes), whereas cells further away from the source face much lower levels (resulting in the induction of low-threshold target genes). Examples from *Drosophila* development are shown, such as the *wingless*/*engrailed* (*wg*/*en*) circuitry in establishing segment polarity and the induction of *senseless* (*sens*) and *distalless* (*dll*) in the wing imaginal disc.

(Bottom right) The power of *Drosophila* genetics resulted in an early map of the Wnt pathway and its core signaling mechanism. It recognized an early role for *porcupine* (PORCN) in the Wnt-producing cell and revealed the order of signaling events in the receiving cells with *frizzled2* (FZD), *dishevelled* (DVL), *zeste-white 3* (GSK3), and *armadillo* (CTNNB1) transducing the signal. Current Wnt-secretion inhibitors block the activities of mammalian PORCN. The precise mode of interaction between Wnt and its receptor was not revealed until the Wnt/Fzd crystal structure was resolved by Claudia Janda and Chris Garcia in 2012.

(Bottom left) Successful purification of active WNT3A protein allowed direct testing of a role for Wnt proteins in balancing stem cell self-renewal and differentiation. Nowadays, Wnt proteins are well recognized as self-renewal factors for many different populations of stem cells.

proto-oncogene (Nusse and Varmus, 1982).

After returning to the Netherlands Cancer Institute, Nusse and coworkers spent the remainder of the decade trying to figure out the identity and function of the *int-1* gene. As a sign of the times, the Division of Virology had been renamed to the Division of Molecular Biology, and Nusse and colleagues were eager to apply the appropriate methods to further investigate *int-1*'s biological function and oncogenic properties. This was not immediately successful: sequencing of the gene did not reveal similarities to other known sequences (although it should be noted that the database at the University of California in San Diego contained only 2,000 of such sequences at the time). It did uncover a stretch of hydrophobic amino acids at the N terminus—now known to be the signal peptide for Wnt protein secretion. Attempts to raise antisera against an *int-1* fusion protein expressed in bacteria did not yield antibodies capable of recognizing the native protein. Transcripts of the *int-1* gene could be detected in embryos, but not in adult animals. An early effort to generate *int-1* transgenic mice (in collaboration with molecular geneticist Anton Berns, who had recently introduced genetically engineered mouse models at the institute)

also failed to provide any clues, as the transgenic animals all died prior to weaning.

In 1986, because of the restricted embryonic expression pattern and given the fact that *int-1* was highly conserved in evolution, the idea arose to clone the *Drosophila* homolog, which could then be used to screen for developmental mutants. This turned out to be a lucky decision: the fly homolog mapped to the same chromosomal position as the segment polarity gene *wingless* (*wg*). Just like that, *int-1* had become the first mammalian oncogene with a known developmental mutant in *Drosophila* and, with that, the first example of an oncogene with a critical role in normal development (Rijsewijk et al., 1987).

With their hands on a fly homolog and access to developmental mutants, exciting new opportunities arose. Attempts to generate antisera against the *wg* protein were successful. Earlier work on *wg* had shown that the mutation acted non-cell autonomously. This, in combination with the protein sequence, suggested that the *wg* gene product could be secreted. Together with Peter Lawrence at Cambridge University, who studied pattern formation in the *Drosophila* embryo, Nusse was able to show the specific location of *wg* in developing larva. Electron microscopy studies clearly revealed the presence of *wg* protein inside small vesicles, as well as in the extracellular space between *wg*-producing cells and neighboring *engrailed* (*en*)-expressing cells. The latter also seemed to have taken up *wg* protein, leaving the authors to conclude that "it seems likely that the *wingless* gene product functions as a paracrine factor that binds to a receptor" (van den Heuvel et al., 1989).

In 1989, Roel Nusse was whisked away by Stanford University to join the newly formed Department of Developmental Biology in the Beckman Center for Molecular and Genetic Medicine and, together with most of his group, left the Netherlands Cancer Institute—something that then-director Piet Borst described as "a major blow," highlighting his "broad knowledge, nose for quality and ability to promote collaborations."

The generation of *wg* transgenic flies turned out more successful than the earlier attempts in mice: the flies presented with an embryonic phenotype that made it possible to screen for suppressor mutations and thereby map the factors responsible for transducing the *wg* signal. Multiple groups used similar approaches at the time, harnessing the full power of *Drosophila* developmental genetics that had been made possible by the Nobel prize-winning work of Christiane Nüsslein-Volhard and Eric Wieschaus. In the early 1980s, they had managed to identify, and then classify, many different segmentation mutants. The *wg* mutant belonged to one of these classes—that of the segment polarity genes (Nüsslein-Volhard and Wieschaus, 1980). Mark Peifer and Eric Wieschaus first proposed that another one of these genes, *armadillo* (*arm*), interacted with the *wg* pathway to control pattern formation in the developing and adult fly (Peifer et al., 1991). By combining different mutants, genetic epistasis experiments soon revealed many more components involved in the *wg* pathway and, more importantly, the order in which they acted. Together, work from the Nusse, Peifer, Perrimon, and Wieschaus labs thus revealed the core working mechanism of what was, by then, becoming known as the Wnt pathway (Clevers and Nusse, 2012; Nusse and Varmus, 2012).

In a savvy display of forward thinking, the few scientists working on *int-1*/*wingless*-related proteins in vertebrates jointly proposed a new nomenclature for *int-1* and its rapidly expanding gene family. Among them were Andrew McMahon and Randall Moon, who had by then begun to identify a large family of *int-1*-related genes in mice and frogs. They had also just introduced a powerful technique to study the activity of *int-1* in early vertebrate development: the famous *Xenopus* axis duplication assay (McMahon and Moon, 1989). The name Wnt (pronounced "wint") was proposed "for the wingless-type MMTV integration site that founded the gene family" (Nusse et al., 1991). It has often been said that behind every great man there's a great woman, and rumor has it that it was in fact Roel Nusse's wife, Betsy, who came up with the suggestion for that name (Nusse and Varmus, 2012).

With a large part of the Wnt signal transduction pathway now mapped out, two main question marks remained. What was the identity of the cell-surface receptor that made cells competent to respond to Wnt proteins, and what happened once the signal reached *arm*? *Drosophila* would again provide one of the answers, when *fz2* was identified as a *wg* receptor in a collaborative effort between the labs of Roel Nusse and Jeremy Nathans (Bhanot et al., 1996). Light on the other question was to be shed from a different corner, representing one of the few holes in the Wnt pathway that were plugged without Roel Nusse's apparent direct involvement.

Armadillo in flies had by then been shown to be homologous to beta-catenin (CTNNB1) in vertebrates, a known component of adherens junctions. While Nusse was occupied with the upstream hunt for the Wnt receptor, scientists over in Europe uncovered an interaction between CTNNB1 and transcription factors of the TCF/LEF family. One of them was Hans Clevers, who had up until then been studying TCF1, a transcription factor critical for T cell development. Another was Walter Birchmeier, whose research had been angled toward the role of E-cadherin, and by association CTNNB1, in suppressing cell invasion and metastasis. Yeast-two-hybrid screens revealed CTNNB1 as a binding partner for human TCF1 and, conversely, led to the discovery of LEF1 as a CTNNB1-interacting protein (Behrens et al., 1996; Molenaar et al., 1996). This not only marked a clear end point for how Wnt signaling was ultimately able to regulate target gene transcription but also gave rise to one of the most robust tools to study WNT/CTNNB1 signaling: the famous TOPFLASH luciferase reporter assay, which contains a multimerized stretch of TCF/LEF binding sites. A couple of years prior, Paul Polakis and others had already shown that CTNNB1 interacted with the tumor suppressor protein APC, thereby firmly consolidating the link between Wnt signaling and cancer. By 1998, the idea of a "destruction complex," responsible for the controlled turnover of CTNNB1 in the absence of a WNT signal, had become established in the rapidly expanding community of Wnt researchers, tying all of the available genetic evidence together into a biochemical model that still very much holds up today (Clevers and Nusse, 2012; Nusse and Varmus, 2012).

Meanwhile, Nusse revisited the problem of Wnt protein purification. Plans to overproduce and purify a bioactive form of the mammalian Wnt1 protein had been made as early as 1988 and had failed miserably. But in 2003, Karl Willert and Roel Nusse finally succeeded to purify active mouse WNT3A protein (Willert et al., 2003). The "trick," as it turned out, was to preserve solubility of the Wnt protein—something that is achieved by including serum in the medium while culturing and by including detergent during the purification and fractionation steps. Otherwise, Wnt proteins, which turned out to be highly hydrophobic as a result of a post-translational lipid modification, will aggregate and become nonfunctional. This important achievement chimed in a new chapter in Nusse's research, which would slowly move away from *Drosophila* and back to studying mammalian tissues over the next decade. It was becoming clear that in addition to controlling the development of complex animal tissues, WNT/CTNNB1 signaling was also critical for maintaining their integrity, suggesting a role in stem cell biology. Indeed, Nusse's lab has shown that purified WNT3A is capable of maintaining self-renewal in many types of stem cells, including pluripotent embryonic stem cells and, signaling a brief return of his research to the tissue in which it all began, those of the mammary epithelium (Zeng and Nusse, 2010). Roel Nusse's recent work has also employed *in vivo* lineage-tracing strategies, using expression of the negative-feedback target gene *Axin2* to mark WNT/CTNNB1-responsive cells, to identify new populations of stem cells in multiple tissues, with a recent focus on hepatocytes and adjacent central vein endothelial cells as a liver stem cell/niche compartment.

It should be evident by now that Roel Nusse is a well-deserving recipient of this year's Gairdner award. Major other breakthroughs have been made possible as a result of his work, which reverberates across the cancer research and stem cell biology fields. Retroviral insertional mutagenesis was used for decades after the initial MMTV screen by Nusse and Varmus to identify new oncogenes, for instance by the labs of Anton Berns and Neil Copeland. Model organisms from across the animal kingdom, including the starlet sea anemone Nematostella and planarian flatworms, have revealed a fundamental role for Wnt signaling in regeneration. Organoid cultures, branded "method of the year" by *Science* magazine in 2017, have transformed basic stem cell research and hold great promise for regenerative medicine. Those cultures, started from adult tissue stem cells, almost invariably require active WNT/CTNNB1 signaling for their long-term maintenance.

One of the most exciting promises is that of using the Wnt pathway as a target for therapeutic intervention. This includes the development of selective inhibitors to block aberrant Wnt signaling in cancer, as well as the development of specific agonists to mobilize self-renewal signaling in stem cells, the latter being aimed at preventing tissue degeneration or promoting injury repair. Using a combination of smart design and protein engineering, the generation of surrogate Wnt agonists is showing great promise for future applications in this area (Janda et al., 2017). The discovery of the *Wnt1* gene as being overexpressed, rather than genetically altered, in mammary tumors opened the door to the idea that a mere change in RNA and protein levels, rather than a clear gain or loss of function, could contribute to tumor formation. This also forms the basis for thinking about developmental signaling pathways as targets for cancer therapy, since tumors might highjack these networks to promote their own growth and differentiation even in the absence of mutations. Efforts to develop Wnt-secretion inhibitors or antibodies interfering with WNT/FZD binding have been successful, but clinical implementation of these drugs still has major challenges to overcome. To prevent detrimental side effects of such a treatment, we need to find a way to block oncogenic Wnt signaling while leaving the physiological processes that depend on it intact. To maximize clinical efficacy, patients who have the best chance of benefiting from the drug need to be selected—something that is more difficult in the absence of a clear genetic mutation.

Of course, as far as Wnt signaling itself is concerned, the WNT/CTNNB1 pathway is just one arm of a much larger network. CTNNB1-independent Wnt signaling activities play a critical role in planar cell polarity, convergent extension and other complex cell movements, including cancer cell invasion and metastasis. Here, a younger generation of researchers is following in the footsteps of people like Randall Moon, Norbert Perrimon, and Paul Adler to continue to solve the mysteries in this exciting field.

Roel Nusse himself will probably be the first to remind us that he does not study Wnt, but stem cells. However, there is no denying that his true legacy is that of an entire field of Wnt signaling research. By all intents and purposes, he has become the *pater familias* of an ever-growing community of scientists who find themselves fascinated by the Wnt pathway—the author of this piece included. As everyone who has ever attended a Wnt meeting knows, the show isn't really over until Roel Nusse has picked up the microphone to thank the organizers, summarize the highlights, and point out a few of the key questions that everyone should go home to study. One other contribution, therefore, should not be left unmentioned. As curator of the Wnt homepage (http://wnt.stanford.edu), Nusse has created an online resource that benefits all researchers in the field, as well as those who are first entering it. Starting a website is one thing. Actively maintaining and updating it for more than 20 years is something different altogether. Frequently cited itself, the Wnt homepage is a valuable repository that also contains information that tends to get left out of scientific publications, such as the traps associated with Wnt protein purification, or, in Nusse's words, "unpublished misery in many labs."

At Stanford, Roel Nusse has received continuous support from the Howard Hughes Medical Institute since 1990. As he celebrates his 70th birthday this year, he continues his efforts to identify common principles of tissue development and maintenance. Hopefully, just like last time, the 70s will turn out to be the start of something beautiful.

ACKNOWLEDGMENTS

I thank Tanne van der Wal for designing the figure and acknowledge funding support from the Dutch Cancer Society (KWF Kankerbestrijding), the Netherlands Organization for Scientific Research (NWO), and the University of Amsterdam. I am

happy to have found access to the old annual scientific reports of the Netherlands Cancer Institute online. I know I have omitted the contributions of many, either by mistake or for stylistic reasons.

REFERENCES

Behrens, J., von Kries, J.P., Kühl, M., Bruhn, L., Wedlich, D., Grosschedl, R., and Birchmeier, W. (1996). Functional interaction of β-catenin with the transcription factor LEF-1. Nature *382*, 638–642.

Bhanot, P., Brink, M., Samos, C.H., Hsieh, J.C., Wang, Y., Macke, J.P., Andrew, D., Nathans, J., and Nusse, R. (1996). A new member of the frizzled family from Drosophila functions as a Wingless receptor. Nature *382*, 225–230.

Clevers, H., and Nusse, R. (2012). Wnt/β-catenin signaling and disease. Cell *149*, 1192–1205.

Janda, C.Y., Dang, L.T., You, C., Chang, J., de Lau, W., Zhong, Z.A., Yan, K.S., Marecic, O., Siepe, D., Li, X., et al. (2017). Surrogate Wnt agonists that phenocopy canonical Wnt and β-catenin signalling. Nature *545*, 234–237.

McMahon, A.P., and Moon, R.T. (1989). Ectopic expression of the proto-oncogene int-1 in Xenopus embryos leads to duplication of the embryonic axis. Cell *58*, 1075–1084.

Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destrée, O., and Clevers, H. (1996). XTcf-3 transcription factor mediates β-catenin-induced axis formation in Xenopus embryos. Cell *86*, 391–399.

Nusse, R., and Varmus, H.E. (1982). Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell *31*, 99–109.

Nusse, R., and Varmus, H. (2012). Three decades of Wnts: A personal perspective on how a scientific field developed. EMBO J. *31*, 2670–2684.

Nusse, R., Brown, A., Papkoff, J., Scambler, P., Shackleford, G., McMahon, A., Moon, R., and Varmus, H. (1991). A new nomenclature for int-1 and related genes: the Wnt gene family. Cell *64*, 231.

Nüsslein-Volhard, C., and Wieschaus, E. (1980). Mutations affecting segment number and polarity in Drosophila. Nature *287*, 795–801.

Peifer, M., Rauskolb, C., Williams, M., Riggleman, B., and Wieschaus, E. (1991). The segment polarity gene armadillo interacts with the wingless signaling pathway in both embryonic and adult pattern formation. Development *111*, 1029–1043.

Rijsewijk, F., Schuermann, M., Wagenaar, E., Parren, P., Weigel, D., and Nusse, R. (1987). The Drosophila homolog of the mouse mammary oncogene int-1 is identical to the segment polarity gene wingless. Cell *50*, 649–657.

van den Heuvel, M., Nusse, R., Johnston, P., and Lawrence, P.A. (1989). Distribution of the wingless gene product in Drosophila embryos: a protein involved in cell-cell communication. Cell *59*, 739–749.

Willert, K., Brown, J.D., Danenberg, E., Duncan, A.W., Weissman, I.L., Reya, T., Yates, J.R., 3rd, and Nusse, R. (2003). Wnt proteins are lipid-modified and can act as stem cell growth factors. Nature *423*, 448–452.

Zeng, Y.A., and Nusse, R. (2010). Wnt proteins are self-renewal factors for mammary stem cells and promote their long-term expansion in culture. Cell Stem Cell *6*, 568–577.