Development 134, 3393-3399 (2007) doi:10.1242/dev.008169

WNTers in La Jolla

Sergei Y. Sokol[1] and Keith A. Wharton, Jr[2]

A 'traditional' Wnt meeting, the first of which occurred over two decades ago as a meeting of the laboratories of Harold Varmus and Roel Nusse, was held at the University of California, San Diego, in June 2007. Organized by Karl Willert, Anthony Wynshaw-Boris and Katherine Jones, the meeting was attended by nearly 400 scientists interested in 'all things Wnt', including Wnt signal transduction mechanisms, and Wnt signaling in evolutionary and developmental biology, stem cell biology, regeneration and disease. Themes that dominated the meeting included the need for precise control over each step of the signal transduction mechanism and developing therapeutics for diseases caused by altered Wnt-signaling.

Introduction

Wnts are a family of signaling proteins that exert a profound influence on cell fate and behavior in all animals thus far studied (Clevers, 2006; Nusse, 2005). Chosen from among nearly 200 abstracts, the presentations were roughly split between talks that probed mechanisms by which Wnt signals activate effector pathways or target genes, and those that explored how Wnt signaling governs higher-order phenomena, including development, regeneration and disease.

Mechanisms of Wnt signaling: sending the signal

Several presentations were devoted to the mechanisms of signal generation by Wnt-secreting cells and signal presentation to responding cells. Recent work from the laboratory of Hendrik Korswagen (Hubrecht Institute, Utrecht, Netherlands) has demonstrated that efficient Wnt production in *Caenorhabditis elegans* requires retromer, an intracellular protein-sorting complex (Coudreuse et al., 2006; Prasad and Clark, 2006). Korswagen and Xinhua Lin (Cincinnati Children's Hospital, OH, USA) reported that *C. elegans* MIG-14 and its *Drosophila* homolog, Wntless (Wls, also known as Sprinter and Evenness interrupted) (Banziger et al., 2006; Bartscherer et al., 2006; Ching and Nusse, 2006; Hausmann et al., 2007), which are conserved transmembrane proteins that are essential for Wnt secretion, require components of the retromer complex for activity. MIG-14 protein levels and Wnt signaling were reduced in the absence of retromer function. Both groups found that the requirement for retromer in Wnt-producing cells in *Drosophila* is bypassed by Wls overexpression. These findings suggest a model in which the rate of Wnt secretion is governed by retromer-dependent recycling of Wls from the plasma membrane back to the Golgi (Fig. 1A).

Lipid modification of Wnt proteins and their low solubility in aqueous environments has raised the issue of how such aggregation-prone molecules transit through tissue to reach distant responding cells. The identification of Wnt-associated proteins, including lipophorin and proteoglycans (Eaton, 2006; Lin, 2004; Panakova et al., 2005), suggests that Wnts are not alone in this challenging task. 'Contaminating' some of the biochemical preparations of a *Drosophila* Wnt protein, as reported by Kim Harnish in Roel Nusse's laboratory (Stanford University, CA, USA), was a member of the Lipocalin family of lipid transport proteins, which are best-known for their roles in transporting small hydrophobic molecules in plants (Grzyb et al., 2006). Harnish reported that Lipocalin-conditioned medium potentiated the activity of Wingless (Wg), a *Drosophila* homolog of the vertebrate protein Wnt1, in cultured cells, whereas Lipocalin depletion had the opposite effect. It will be of great interest to establish a role for lipocalins in regulating Wnt signaling in vivo.

Signal reception and intracellular transduction

How Wnt ligand-receptor interactions elicit intracellular signaling remains a subject of intense study. 'Canonical' Wnt signaling leads to the accumulation of β-catenin and the altered transcription of target genes, but many 'non-canonical' Wnt pathways are being increasingly recognized. In current models, canonical signaling is initiated by Wnt jointly engaging Frizzled (Fz) and low density lipoprotein-related proteins 5/6 (Lrp5/6, also known as Arrow – FlyBase) receptors, leading to Lrp5/6 phosphorylation (Cadigan and Liu, 2006; He et al., 2004). Dishevelled (Dsh, Dvl in vertebrates) is a family of scaffolding proteins that bind several Wnt signaling components and are required for Wnt signal transmission, but how Dsh inhibits the complex that promotes β-catenin destruction remains enigmatic (Wallingford and Habas, 2005). Xi He (Children's Hospital, MA, USA) reported that mammalian cells that lack Dvl function had reduced levels of Wnt-induced Lrp6 phosphorylation, indicating that Dvl might act upstream of Lrp5/6 in canonical signaling. Together with work recently published by the group of Marcel Wehrli (Oregon Health Sciences University, OR, USA) (Baig-Lewis et al., 2007), the current data suggest that canonical signaling proceeds through discrete initiation and amplification steps mediated by the stepwise assembly of macromolecular complexes that include Wnt ligands, their receptors, Dvl, Axin and the protein kinases Casein kinase I (CK1) and Glycogen Synthase Kinase 3 (GSK3, also known as Shaggy – FlyBase) that phosphorylate Lrp5/6 (Fig. 1B). These findings extend the model for signaling via Lrp5/6-mediated Axin sequestration (Mao et al., 2001; Tamai et al., 2004) and further support the idea that efficient Wnt signaling requires the assembly of a large protein signaling complex, the Wnt 'signalosome' (Bilic et al., 2007) (Fig. 1B).

Attracting much recent attention are a family of four secreted proteins termed R-spondins (RSpo), which can regulate canonical Wnt signaling in several tissues and which have been implicated in human disease (Kazanskaya et al., 2004; Kim et al., 2006; Wei et al., 2007). One possibility is that R-Spondins represent a class of non-Wnt ligands that bind and activate Wnt receptors in a fashion analogous to Wnt proteins themselves (Nam et al., 2006; Wei et al., 2007), but Minke Binnerts (Nuvelo, CA, USA) presented evidence that RSpo1 activates the canonical signaling pathway by antagonizing the activity of the extracellular Wnt inhibitor dickkopf 1 (Dkk1) in mammalian cells. Binding of RSpo1 to kringle containing transmembrane protein 1 (Kremen1), a transmembrane protein that associates with Dkk1 (Mao et al., 2002), results in

[1]Department of Molecular, Cell and Developmental Biology, Box 1020, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, USA.
[2]Laboratory of Molecular Pathology, Departments of Pathology and Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390-9072, USA.

e-mails: sergei.sokol@mssm.edu; keith.wharton@utsouthwestern.edu

increased Lrp6 levels at the cell surface. Thus, RSpo1 might lower the activation threshold for Wnt proteins by relieving the inhibition imposed on Wnt signaling by Dkk1 (Binnerts et al., 2007).

Non-canonical Wnt pathways mediate important steps in morphogenesis, such as convergent extension during vertebrate gastrulation, but their relationship to the canonical signaling, which is often triggered by the same ligand, remains understudied.

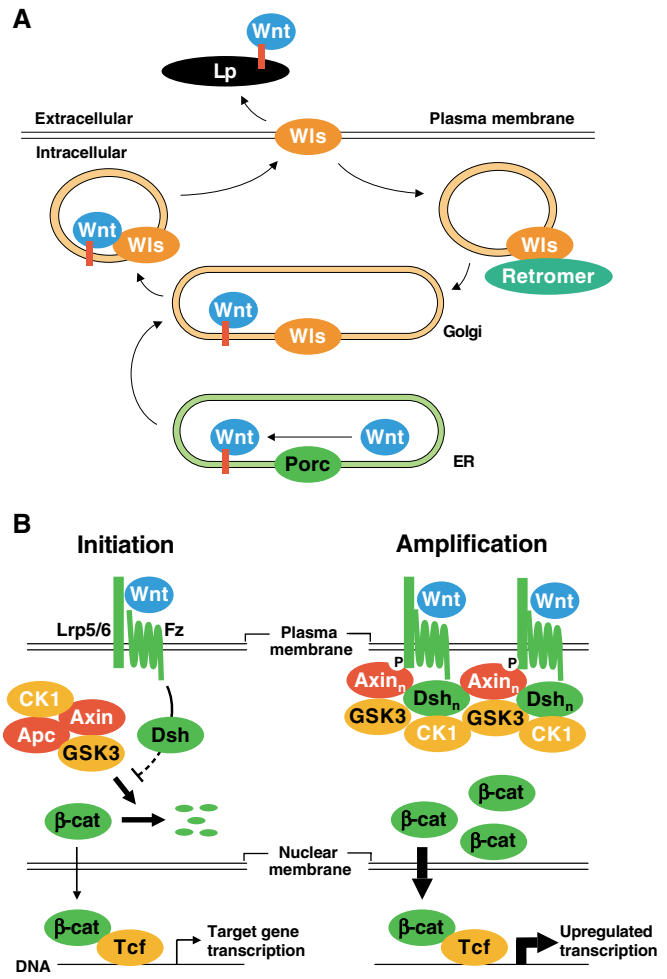

Fig. 1. Models for Wnt signal production and reception. (**A**) Wnt signal production. Wnt is palmitoylated (red rectangle) by Porcupine (Porc) in the lumen of the endoplasmic reticulum (ER). Upon reaching the Golgi, Wntless (also known as Evenness interrupted and Sprinter, and as MIG-14 in *C. elegans*; Wls, orange) associates with Wnt in vesicles bound for secretion (arrows), leading to extracellular association of Wnt with lipoprotein particles (Lp). The retromer complex recycles Wls at the plasma membrane back to the Golgi. (**B**) A two-step model for Wnt signal reception. In the absence of a Wnt signal, the labile β-catenin molecule is phosphorylated by a complex consisting of Axin, Apc, and the kinases CK1 and GSK3β, and is then rapidly degraded. Signaling is initiated (left) by association of Wnt with its co-receptors Lrp5/6 and Fz at the plasma membrane, leading to the Dsh-dependent partial inhibition of β-catenin degradation and its translocation through the nuclear membrane. In the nucleus, β-catenin activates target gene transcription by associating with Tcf. Signal amplification (right) occurs via GSK3- and CK1-dependent phosphorylation (P) of Lrp5/6, leading to further recruitment of Axin and Dsh oligomers (n) to form the 'Wnt signalosome', causing increased accumulation of nuclear β-catenin and further upregulation of target gene transcription. See text for references.

Alexandra Schambony in Doris Wedlich's laboratory (University of Karlsruhe, Karlsruhe, Germany) discussed the involvement of vertebrate Ror2, a receptor tyrosine kinase, in Wnt5a-mediated non-canonical signaling. Schambony reported that depletion of either Wnt5a or Ror2 resulted in defective convergent extension movements in *Xenopus* embryos (Fig. 2A,B), a finding that might be related to the dependence on Ror2 for Wnt5a-induced cell migration and filopodia dynamics in cultured cells (Nishita et al., 2006). Schambony demonstrated that a major target of Wnt5a/Ror2 signaling was transcriptional upregulation of the *Xpapc* (*paraxial protocadherin*) gene via a pathway involving PI3-kinase, cdc42 and Map-kinase-dependent activation of Atf2/Jun transcriptional complexes (Schambony and Wedlich, 2007). This report raises the possibility that targeting transcription might be a general property of noncanonical Wnt pathways. Wnt5a has long been recognized for its ability to inhibit canonical Wnt signaling (Torres et al., 1996). Amanda Mikels from the Nusse laboratory reported that Ror2 mediates Wnt5a-dependent inhibition of canonical β-catenin signaling (Mikels and Nusse, 2006), and suggested that the molecular basis of some cases of human brachydactyly (congenitally short digits) might be due to reduced Wnt5a/Ror2 signaling and hence elevated canonical Wnt signaling. It remains to be demonstrated whether Wnt5a-mediated inhibition of canonical signaling occurs via a signaling crosstalk or whether it requires transcriptional responses.

Recent studies in mice have revealed an unsuspected link between the cilium, a microtubule-based cell protrusion, and Hedgehog (Hh) signaling (Eggenschwiler and Anderson, 2006; Huangfu and Anderson, 2005). Functional interactions between Dvl and the ciliary protein inversin (Invs) (Simons et al., 2005) suggest that similar links might exist between cilia and Wnt signaling (Oishi et al., 2006; Park et al., 2006). Kevin Corbit from Jeremy Reiter's laboratory [University of California (UC) at San Francisco, CA, USA] observed upregulated canonical Wnt signaling in mouse embryos and cells lacking the kinesin Kif3a, a motor protein required for cilia formation (Marszalek et al., 1999). Cells lacking cilia contained stabilized β-catenin and showed increased CK1-dependent phosphorylation of Dvl, in support of a model in which cilia normally restrain the ability of CK1 to activate Wnt signaling. In a related presentation, Vera Voronina from Randy Moon's laboratory (University of Washington, WA, USA) reported that mice deficient in Chibby (Cby), an antagonist of β-catenin (Takemaru et al., 2003), lack mucociliary transport in sinuses, suggesting that hyperactive Wnt signaling caused by Cby deficiency might interfere with cilia function in vivo.

Signaling in the nucleus

Precise regulation of Wnt signal-dependent gene expression requires a carefully orchestrated balance between activation and repression. Although complexes between β-catenin and the Tcf family of high mobility group (HMG)-domain transcription factors remain the commonly accepted link between Wnt signals and target gene activation, some developmental functions of Tcf proteins might be independent of β-catenin. Mice mutant in one of the four Tcf paralogs, *Tcf3*, die early in embryogenesis with partial axis duplications and gastrulation defects, presumably due to derepression of the crucial target gene *Nanog* in cells fated to give rise to the primitive streak (Merrill et al., 2004; Pereira et al., 2006). Bradley Merrill (University of Illinois, IL, USA) showed that mice homozygous for a *Tcf3* allele that lacks the β-catenin binding sequence form a normal primitive streak, suggesting that Tcf3–β-catenin interactions are not required for *Nanog* repression

and primitive streak formation. Because Wnt proteins are expressed in preimplantation-stage embryos and are essential for primitive streak formation following implantation (Kemp et al., 2005; Liu et al., 1999; Wang et al., 2004), the question of which Tcf proteins transduce Wnt signals in early mouse embryos remains important.

Non-overlapping roles for Tcf proteins in different tissues might in principle be due to tissue-specific expression patterns or due to intrinsic differences in protein function. Two Tcf proteins, Tcf1 and Tcf4, exist as C-terminal splice variants (known as the E-tail) with distinct DNA-binding and functional properties (Arce et al., 2006).

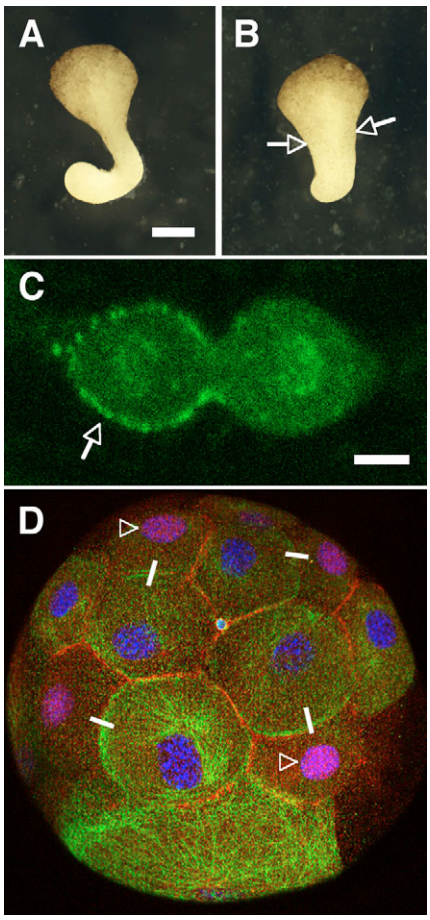

Fig. 2. Wnt signaling in morphogenesis and cell polarity. (**A**,**B**) A requirement for Ror2 in non-canonical Wnt signaling. Elongation and constriction of Keller explants (dorsal tissue from *Xenopus* gastrulae) used as a model of non-canonical Wnt5a signaling (see text). (A) Uninjected explant. (B) Ror2 morpholino-injected explant shows lack of constriction (arrows) during convergent extension movements. Image courtesy of Alexandra Schambony. (**C**) Asymmetric WRM-1-GFP localization during telophase in the V5.p cell of the *C. elegans* larva. Note cortical localization of WRM-1 in the newly forming cell on the left (arrow) and more-pronounced nuclear localization in its sibling cell on the right. Image courtesy of Kota Mizumoto. (**D**) Asymmetric β-catenin localization in a 16-cell *Platynereis dumerilii* embryo. Animal pole view; α-tubulin (green), and histone (blue). Sister cells are connected by white bars. Notice that β-catenin (red) is present at high levels in the nuclei of vegetal-pole sister cells (arrowheads, magenta color), but at low levels in the nuclei of animal-pole sisters. Image courtesy of Stephan Schneider. See text for references. Scale bars: ~150 μm in A for A,B; 2 μm in C.

Marian Waterman (UC Irvine, CA, USA) showed that the E-tail encodes a novel sequence-specific DNA-binding domain that recognizes a GC-rich consensus sequence, thereby imposing additional specificity upon recognition of the core Wnt response element by the Tcf HMG domain. Her group found that the E-tail is essential for Tcf1 and Tcf4 regulation of *Lef1* transcription and for colon cancer cell proliferation, and thus might be involved in the activation of a subset of Wnt target genes that control cell growth.

Although initial studies suggested that Cby represses Wnt signaling by competing with β-catenin for Tcf binding (Takemaru et al., 2003), Ken-Ichi Takemaru (State University of New York at Stony Brook, NY, USA) proposed an additional mechanism for Cby action. Using mass spectrometry, his group identified 14-3-3 proteins as candidate Cby-binding partners. 14-3-3 proteins are a family of small, highly conserved proteins that are best-known for their roles in binding phosphorylated protein substrates and promoting their nuclear export (Dougherty and Morrison, 2004). Cby–14-3-3 binding enhances cytoplasmic sequestration of β-catenin in a manner dependent on the phosphorylation of a serine residue in the 14-3-3-binding domain of Cby. These observations suggest that Cby in concert with 14-3-3 inhibits signaling by facilitating nuclear export of β-catenin.

Wnt signaling and cell polarity

Wnt signals regulate cell polarity and asymmetric cell division, and some signaling components distribute in an asymmetric fashion during signaling events (Fig. 2C,D), but whether such localizations are a cause or a consequence of signaling remains controversial. Initial studies of Wnt signaling in *C. elegans* concerned the Wnt-dependent nuclear export of the Tcf homolog POP-1 from the E blastomere, closer to the source of Wnt, but not from the more distant sister MS blastomere, at the eight-cell stage (Lin et al., 1998). Intriguingly, transiently reduced levels of nuclear POP-1 are observed in posterior daughter cells of nearly all subsequent cell divisions that are oriented along the anteroposterior (AP) axis, whereas SYS-1, a β-catenin homolog, is distributed in a complementary pattern (Huang et al., 2007; Phillips et al., 2007). By contrast, in vertebrate development, as originally demonstrated in *Xenopus*, nuclear localization of β-catenin is first observed in a population of dorsal cells prior to gastrulation (Larabell et al., 1997; Schneider et al., 1996). Similarly, nuclear β-catenin is found in specific groups of cells in early sea urchin (Logan et al., 1999; Wikramanayake et al., 1998) and sea anemone (Wikramanayake et al., 2003) embryos. Stephan Schneider from Bruce Bowerman's laboratory (University of Oregon, OR, USA) examined the localization of β-catenin during early animal-vegetal axis-oriented cell divisions in the polychaete annelid *Platynereis dumerilii* (Schneider and Bowerman, 2007). Remarkably, nuclear β-catenin levels were higher in vegetal daughter cells as compared with animal daughter cells (Fig. 2D). Treatment with GSK3 inhibitors that block β-catenin degradation promoted nuclear accumulation of β-catenin in animal daughter cells and resulted in animal-to-vegetal cell fate transformations. The observed asymmetries are proposed to constitute an ancient binary cell fate-specification mechanism, retained in annelid and nematode worms, that has been modified during evolution to regulate cell fate in populations of cells in other metazoans.

The dual functions of β-catenin as a Wnt effector and a component of the adherens junction have raised the question of how the different pools of β-catenin affect signaling (Nelson and Nusse, 2004). In *C. elegans*, the four known β-catenin homologs have evolved distinct signaling and adhesion functions, some of which

appear to be crucial for Wnt-directed asymmetric cell divisions. Kota Mizumoto from Hitoshi Sawa's laboratory (RIKEN, Kobe, Japan) showed that forced expression of a membrane-anchored form of the β-catenin homolog WRM-1 inhibits Wnt signaling and localization of endogenous WRM-1 to the nucleus in a manner dependent on *APR-1*, a *C. elegans* homolog of the vertebrate adenomatous polyposis coli (*APC*) gene (Mizumoto and Sawa, 2007). Their data suggest a model whereby Wnt-dependent asymmetric cortical and nuclear localizations of crucial signaling components direct polarized cell divisions and subsequent fate determination of each daughter cell (Fig. 2C). Although a role for Wnt signaling in the asymmetric division of vertebrate cells remains largely inferred, a poster by Christophe Marcelle (Developmental Biology Institute of Marseille, Luminy, France) showed that Wnt11 acts as a directional cue to orient embryonic myocytes during skeletal muscle morphogenesis in chick embryos.

Wnt signaling in development

During mouse development, *Wnt3a* is expressed in the posterior region of the early- to mid-gestation embryo and is essential for mesoderm specification and somitogenesis (Takada et al., 1994). Bill Dunty from Terry Yamaguchi's group (National Cancer Institute, MD, USA) used microarrays to identify a Wnt3a-target gene in mouse termed mesogenin 1 (Yoon and Wold, 2000), which encodes a basic helix-loop-helix (bHLH) transcription factor that promotes mesoderm maturation by acting as a feedback suppressor to limit the domain of *Wnt3a* expression.

Wnt signaling plays multiple sequential roles in cardiogenesis (Hamblet et al., 2002; Tzahor, 2007). Alexandra Klaus from Walter Birchmeier's laboratory (Max-Delbrueck Center, Berlin, Germany) demonstrated that Bmp and Wnt signaling have to be precisely timed for induction of the mouse primary and secondary heart fields, respectively. Jianbo Wang and Leah Etheridge (Wynshaw-Boris laboratory, UC San Diego, CA, USA) discussed the role of the three mouse Dvl genes in cardiac development. Using a BAC transgenic rescue assay, Wang showed that the conotruncal heart defects in $Dvl1^{-/-}$ $Dvl2^{-/-}$ mice are due to defective non-canonical Wnt signaling in the secondary heart field (which normally gives rise to the right atrium, ventricle and outflow tract), but not in the cardiac neural crest. Etheridge used green fluorescent protein (GFP)-tagged Dvl transgenes to rescue Dvl-mutant phenotypes and observed distinct subcellular localizations for the different Dvl proteins in the cochlea, an organ that requires non-canonical Wnt signaling for the proper alignment of stereocilia. These observations suggest that the three mammalian Dvl proteins might play unique roles in specific signaling events during vertebrate development.

In the nervous system, Wnts have been shown to regulate neuronal polarity and migration, axon pathfinding, and synaptogenesis (Hilliard and Bargmann, 2006; Lyuksyutova et al., 2003; Pan et al., 2006; Salinas, 2005; Speese and Budnik, 2007; Zhang et al., 2007; Zou, 2004). Jason Kennerdell from Cori Bargmann's laboratory (Rockefeller University, NY, USA) showed that CWN-2, one of the five *C. elegans* Wnts, acts with both Fz and ROR (receptor tyrosine kinase-like orphan receptor) receptors to regulate the position of the pharyngeal nerve ring. Previous work has suggested that Wnt signaling promotes synaptogenesis (Salinas, 2005; Speese and Budnik, 2007), but Kang Shen (Stanford University, CA, USA) showed that Wnt signaling inhibits en passant synaptogenesis of the DA9 neuron in *C. elegans* (Klassen and Shen, 2007). Scott Clark (New York University, NY, USA) reported that the RING-finger protein PLR-1 downregulates Wnt signaling by reducing the cell surface levels of Frizzled during neuronal development. Yimin Zou (UC San Diego, CA, USA) presented data suggesting that PI3 kinase and atypical protein kinase C (aPKC) mediate Wnt4 signaling in migrating commissural axon growth cones after crossing the midline in the vertebrate neural tube. In support of these observations, a recent study has suggested that the interaction of Dvl and aPKC mediates Wnt signaling in neuronal polarity (Zhang et al., 2007).

Several presentations explored the role of Wnt signaling in vertebrate eye development. Fumi Kubo from Shinichi Nakagawa's laboratory (RIKEN, Wako, Japan) identified Hairy1, a bHLH transcription factor whose transcription can be regulated by the Notch pathway (Davis and Turner, 2001), as a target of Wnt2b that maintains stem cells in the ciliary marginal zone of the developing eye (Kubo et al., 2005). Pygopus (Pygo) is required for all β-catenin-dependent transcription in *Drosophila* via bridging interactions with Bcl9 (also known as legless, Lgs) (Belenkaya et al., 2002; Kramps et al., 2002; Parker et al., 2002; Thompson et al., 2002). By contrast, many tissues that require Wnt signaling develop normally in mice that lack the two *pygo* homologs, *pygo1* and *pygo2* (Li et al., 2007; Schwab et al., 2007). Birchmeier's and Richard Lang's (University of Cincinnati, OH, USA) groups demonstrated that the microphthalmia seen in $pygo2^{-/-}$ mouse embryos correlates with a lack of *Pax6* expression and this function of Pygo2 appears to be Wnt-independent (Song et al., 2007). Li Li in Birchmeier's laboratory suggested that the small-eye phenotype of $pygo2^{-/-}$ mutants is due to a failure of *pygo2* to activate the extracellular Wnt inhibitor Sfrp2 in the eye field.

Stem cells and regeneration

Wnts are implicated in stem cell maintenance and are required for tissue regeneration. In the keynote address, Hans Clevers (Hubrecht Institute, Utrecht, Netherlands) described his laboratory's characterization of Wnt/Tcf-regulated target genes in intestinal development and homeostasis using a combination of chromatin immunoprecipitation and microarray approaches. Although thousands of Tcf-binding sites exist on chromatin, only a few of them correspond to bona fide target genes. One target, *Gpr49* (also known as *Lgr5* – Mouse Genome Informatics), encodes an orphan G-protein-coupled receptor that is expressed in colon cancers and appears to mark intestinal stem cells. Lineage tracing of *Gpr49*-expressing cells using *Cre*-based recombination technology resulted in the long-term labeling of whole intestinal crypts, supporting a model in which individual crypts can be maintained and renewed by the progeny of a single stem cell at the base of the crypt. In a related technical advance, Calvin Kuo (Stanford University, CA, USA) described his laboratory's long-term culture (>3 months) of intestinal stem cells.

Several presentations concerned the Wnt-dependent regulation of stem cell self-renewal and tissue regeneration. Arial Zeng from the Nusse laboratory showed that reporter activity for the Wnt target $Axin2^{lacZ}$ enriches for self-renewal in mammary stem cell preparations. Presentations from Wolfram Goessling and Trista North from Len Zon's laboratory (Children's Hospital, MA, USA) discussed the synergy between the prostaglandin and Wnt/β-catenin pathways in hematopoietic stem cell renewal (North et al., 2007) and in liver regeneration. The data suggest that the reported effects of prostaglandins on morphogenesis and inhibition of cancer might be mediated by Wnt signaling (Castellone et al., 2005; Cha et al., 2006). Yasuhiko Kawakami from the Izpisua-Belmonte laboratory (Salk Institute, CA, USA) discussed his studies that demonstrate a crucial role for Wnt/β-catenin signaling in limb regeneration across vertebrate evolution (Kawakami et al., 2006). Growth and

regeneration in vertebrate limbs require interactions between ectoderm and mesenchyme that limit chondrogenesis to those cells distant from surface ectoderm. Lilia Topol from Yingzi Yang's laboratory [National Institutes of Health (NIH), MD, USA] discussed a dual mechanism used by the pro-chondrogenic HMG-domain transcription factor Sox9 to inhibit the anti-chondrogenic effects of the Wnt/β-catenin pathway (Akiyama et al., 2004; Hill et al., 2005). Derk Ten Berge from the Nusse laboratory proposed that an ectodermal Wnt signal suppresses *Sox9* expression in underlying mesenchyme, and hypothesized that the extent of growth and differentiation during limb development might be predicted from the range of Wnt3a activity in cultured limb explants.

Diseases and therapeutics

Despite the growing list of Wnt signaling-associated diseases, therapies that rationally target Wnt signaling or its known targets have yet to make a clinical impact. Several presentations and posters described screens for small molecules that affect Wnt signaling. Michael Kahn (University of Southern California, CA, USA) described two inhibitors that disrupt the interaction between β-catenin and the CREB-binding protein (CBP or p300) histone acetyl transferase coactivators, the choice of which determines whether embryonic stem (ES) cells differentiate or proliferate, respectively (Ma et al., 2005). Interestingly, blocking the p300/β-catenin interaction with one of these molecules supported the proliferation of ES cells in culture, without serum or added growth factors, in a Wnt3a-dependent fashion (Miyabayashi et al., 2007). To avoid tumor formation following therapeutic stem cell implantation, the balance between proliferation and differentiation of implanted cells needs to be highly coordinated. In this regard, Ernest Arenas (Karolinska Institute, Stockholm, Sweden) successfully differentiated fetal mesencephalic stem cells into dopaminergic (DA) neurons in a Wnt5a-dependent manner (Bryja et al., 2007) and demonstrated their efficacy when transplanted into a mouse model of Parkinson's disease. These proof-of-principle studies are required to justify future stem cell transplants intended to replace lost DA neurons in Parkinson's patients (Parish and Arenas, 2007).

Vanessa Bres in Katherine Jones' laboratory (UC San Diego, CA, USA) discussed the cooperation of Wnt and Notch signaling pathways in human breast and colon cancer. Charlotta Lindvall in Bart Williams' laboratory (Van Andel Research Institute, MI, USA) showed that rapamycin-dependent inhibition of the target of rapamycin (TOR) pathway, as well as loss of Lrp5, inhibits tumor cell growth in a mouse mammary tumor virus (MMTV)-Wnt mouse breast cancer model, in line with the known tumorigenic properties of the TOR pathway (Bjornsti and Houghton, 2004; Hay and Sonenberg, 2004). Predisposition to Wilms tumor, a childhood renal cancer with elevated Wnt signaling, is often due to mutant WT1 or WTX (FAM123B) proteins, the latter of which was recently shown to associate with the β-catenin 'destruction complex' (Major et al., 2007; Rivera and Haber, 2005; Rivera et al., 2007). However, the mechanism by which WT1 suppresses Wilms tumor formation or canonical Wnt signaling remains unclear. Myoung Shin Kim in Sean Lee's laboratory (NIH, MD, USA) searched for WT1 targets (Kim et al., 2007) and identified a novel gene product similar to the Dvl-binding protein IDAX (CXXC4) (Hino et al., 2001), which might mediate WT1-dependent suppression of Wnt/β-catenin signaling. Catriona Jamieson (UC San Diego, CA, USA) described a population of cancer stem cells in blast-phase chronic myeloid leukemia (CML) with elevated Wnt/β-catenin signaling, presumably due to secondary mutations in the machinery that promote β-catenin degradation (Jamieson et al., 2004). In some CML patients in blast crisis, she identified unique splice mutations in the *GSK3B* gene that might be responsible for elevated Wnt/β-catenin signaling, but whether the mutations act in a loss-of-function or dominant-negative manner remains to be determined.

Given the widespread role of Wnt/β-catenin signaling in stem cell and tissue homeostasis, specificity of therapeutic targeting remains a formidable challenge. Venita DeAlmeida from Paul Polakis' group at Genentech (CA, USA) discussed their efforts to generate an extracellular Wnt inhibitor with optimal pharmacokinetic properties, efficacy and minimal side effects (DeAlmeida et al., 2007). The extracellular domain of the Fz8 receptor fused to the Fc portion of human immunoglobulin remains relatively stable when injected into mice ($t_{1/2}$~4 days). The fusion protein significantly inhibited growth of MMTV-Wnt1 mammary tumors, as well as tumors derived from PA-1 and NTera2 teratoma cell lines, whose growth in vitro requires autocrine Wnt signaling. Remarkably, at doses in which the chimeric molecule suppressed the growth of MMTV-Wnt1 and NTera2 tumors in nude mice, there were no adverse effects on β-catenin levels or on the histology of intestine or hair follicles, two tissues whose constant turnover requires Wnt signaling. These data provide hope that Wnt signaling might someday be selectively targeted in diseased cells and tissues with minimal host toxicity.

Conclusion

As co-discoverer of Wnt proteins a quarter century ago, Roel Nusse closed the meeting by commenting that the Wnt field continues to be characterized by a 'vigorous and rich' array of science. The meeting demonstrated the need to further understand the context-dependence of diverse Wnt pathways and the molecular bases by which they integrate with other signaling processes in the cell. The breadth of Wnt-related investigations and their impact in modern biomedical science is truly amazing. We anticipate that the growing significance of 'all things Wnt', as evidenced by over 6,000 Wnt-related references in PubMed, ensures that future Wnt meetings will be as exciting as the one in La Jolla.

We thank Karl Willert, Anthony Wynshaw-Boris and Katherine Jones for a flawless meeting organization and a stimulating scientific program; individuals mentioned in the review for their feedback on the manuscript and for their permission to discuss unpublished data; and all meeting attendees for lively scientific discussion. We regret that due to space constraints we were unable to cover many interesting presentations. We acknowledge funding by March of Dimes Birth Defects Foundation (to S.Y.S.) and the NIH (to S.Y.S. and K.A.W.). K.A.W. is a W. W. Caruth, Jr, Scholar in Biomedical Science at UT Southwestern.

References

Akiyama, H., Lyons, J. P., Mori-Akiyama, Y., Yang, X., Zhang, R., Zhang, Z., Deng, J. M., Taketo, M. M., Nakamura, T., Behringer, R. R. et al. (2004). Interactions between Sox9 and β-catenin control chondrocyte differentiation. *Genes Dev.* **18**, 1072-1087.

Arce, L., Yokoyama, N. N. and Waterman, M. L. (2006). Diversity of LEF/TCF action in development and disease. *Oncogene* **25**, 7492-7504.

Baig-Lewis, S., Peterson-Nedry, W. and Wehrli, M. (2007). Wingless/Wnt signal transduction requires distinct initiation and amplification steps that both depend on Arrow/LRP. *Dev. Biol.* **306**, 94-111.

Banziger, C., Soldini, D., Schutt, C., Zipperlen, P., Hausmann, G. and Basler, K. (2006). Wntless, a conserved membrane protein dedicated to the secretion of Wnt proteins from signaling cells. *Cell* **125**, 509-522.

Bartscherer, K., Pelte, N., Ingelfinger, D. and Boutros, M. (2006). Secretion of Wnt ligands requires Evi, a conserved transmembrane protein. *Cell* **125**, 523-533.

Belenkaya, T. Y., Han, C., Standley, H. J., Lin, X., Houston, D. W., Heasman, J. and Lin, X. (2002). *pygopus* encodes a nuclear protein essential for wingless/Wnt signaling. *Development* **129**, 4089-4101.

Bilic, J., Huang, Y. L., Davidson, G., Zimmermann, T., Cruciat, C. M., Bienz, M. and Niehrs, C. (2007). Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation. *Science* **316**, 1619-1622.

Binnerts, M. E., Kim, A. K., Bright, J. M., Patel, S. J., Tran, K., Zhou, M., Leung, J. M., Liu, Y., Lomas, W. E., III, Dixon, M. et al. (2007). R-Spondin1 regulates Wnt signaling by inhibiting internalization of LRP6. *Proc. Natl. Acad. Sci. USA* In press.
Bjornsti, M. A. and Houghton, P. J. (2004). The TOR pathway: a target for cancer therapy. *Nat. Rev. Cancer* **4**, 335-348.
Bryja, V., Schulte, G., Rawal, N., Grahn, A. and Arenas, E. (2007). Wnt-5a induces Dishevelled phosphorylation and dopaminergic differentiation via a CK1-dependent mechanism. *J. Cell Sci.* **120**, 586-595.
Cadigan, K. M. and Liu, Y. I. (2006). Wnt signaling: complexity at the surface. *J. Cell Sci.* **119**, 395-402.
Castellone, M. D., Teramoto, H., Williams, B. O., Druey, K. M. and Gutkind, J. S. (2005). Prostaglandin E2 promotes colon cancer cell growth through a G_s-axin-β-catenin signaling axis. *Science* **310**, 1504-1510.
Cha, Y. I., Kim, S. H., Sepich, D., Buchanan, F. G., Solnica-Krezel, L. and DuBois, R. N. (2006). Cyclooxygenase-1-derived PGE2 promotes cell motility via the G-protein-coupled EP4 receptor during vertebrate gastrulation. *Genes Dev.* **20**, 77-86.
Ching, W. and Nusse, R. (2006). A dedicated Wnt secretion factor. *Cell* **125**, 432-433.
Clevers, H. (2006). Wnt/β-catenin signaling in development and disease. *Cell* **127**, 469-480.
Coudreuse, D. Y., Roel, G., Betist, M. C., Destree, O. and Korswagen, H. C. (2006). Wnt gradient formation requires retromer function in Wnt-producing cells. *Science* **312**, 921-924.
Davis, R. L. and Turner, D. L. (2001). Vertebrate hairy and Enhancer of split related proteins: transcriptional repressors regulating cellular differentiation and embryonic patterning. *Oncogene* **20**, 8342-8357.
DeAlmeida, V. I., Miao, L., Ernst, J. A., Koeppen, H., Polakis, P. and Rubinfeld, B. (2007). The soluble wnt receptor Frizzled8CRD-hFc inhibits the growth of teratocarcinomas in vivo. *Cancer Res.* **67**, 5371-5379.
Dougherty, M. K. and Morrison, D. K. (2004). Unlocking the code of 14-3-3. *J. Cell Sci.* **117**, 1875-1884.
Eaton, S. (2006). Release and trafficking of lipid-linked morphogens. *Curr. Opin. Genet. Dev.* **16**, 17-22.
Eggenschwiler, J. T. and Anderson, K. V. (2006). Cilia and developmental signaling. *Annu. Rev. Cell Dev. Biol.* **23**, doi:10.1146/annurev.cellbio.23.090506.123249.
Grzyb, J., Latowski, D. and Strzalka, K. (2006). Lipocalins – a family portrait. *J. Plant Physiol.* **163**, 895-915.
Hamblet, N. S., Lijam, N., Ruiz-Lozano, P., Wang, J., Yang, Y., Luo, Z., Mei, L., Chien, K. R., Sussman, D. J. and Wynshaw-Boris, A. (2002). Dishevelled 2 is essential for cardiac outflow tract development, somite segmentation and neural tube closure. *Development* **129**, 5827-5838.
Hausmann, G., Banziger, C. and Basler, K. (2007). Helping Wingless take flight: how WNT proteins are secreted. *Nat. Rev. Mol. Cell Biol.* **8**, 331-336.
Hay, N. and Sonenberg, N. (2004). Upstream and downstream of mTOR. *Genes Dev.* **18**, 1926-1945.
He, X., Semenov, M., Tamai, K. and Zeng, X. (2004). LDL receptor-related proteins 5 and 6 in Wnt/β-catenin signaling: arrows point the way. *Development* **131**, 1663-1677.
Hill, T. P., Spater, D., Taketo, M. M., Birchmeier, W. and Hartmann, C. (2005). Canonical Wnt/β-catenin signaling prevents osteoblasts from differentiating into chondrocytes. *Dev. Cell* **8**, 727-738.
Hilliard, M. A. and Bargmann, C. I. (2006). Wnt signals and frizzled activity orient anterior-posterior axon outgrowth in C. elegans. *Dev. Cell* **10**, 379-390.
Hino, S., Kishida, S., Michiue, T., Fukui, A., Sakamoto, I., Takada, S., Asashima, M. and Kikuchi, A. (2001). Inhibition of the Wnt signaling pathway by Idax, a novel Dvl-binding protein. *Mol. Cell. Biol.* **21**, 330-342.
Huang, S., Shetty, P., Robertson, S. M. and Lin, R. (2007). Binary cell fate specification during *C. elegans* embryogenesis driven by reiterated reciprocal asymmetry of TCF POP-1 and its coactivator {β}-catenin SYS-1. *Development* **134**, 2685-2695.
Huangfu, D. and Anderson, K. V. (2005). Cilia and Hedgehog responsiveness in the mouse. *Proc. Natl. Acad. Sci. USA* **102**, 11325-11330.
Jamieson, C. H., Ailles, L. E., Dylla, S. J., Muijtjens, M., Jones, C., Zehnder, J. L., Gotlib, J., Li, K., Manz, M. G., Keating, A. et al. (2004). Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N. Engl. J. Med.* **351**, 657-667.
Kawakami, Y., Rodriguez Esteban, C., Raya, M., Kawakami, H., Marti, M., Dubova, I. and Izpisua Belmonte, J. C. (2006). Wnt/β-catenin signaling regulates vertebrate limb regeneration. *Genes Dev.* **20**, 3232-3237.
Kazanskaya, O., Glinka, A., del Barco Barrantes, I., Stannek, P., Niehrs, C. and Wu, W. (2004). R-Spondin2 is a secreted activator of Wnt/β-catenin signaling and is required for *Xenopus* myogenesis. *Dev. Cell* **7**, 525-534.
Kemp, C., Willems, E., Abdo, S., Lambiv, L. and Leyns, L. (2005). Expression of all Wnt genes and their secreted antagonists during mouse blastocyst and postimplantation development. *Dev. Dyn.* **233**, 1064-1075.
Kim, H. S., Kim, M. S., Hancock, A. L., Harper, J. C., Park, J. Y., Poy, G., Perantoni, A. O., Cam, M., Malik, K. and Lee, S. B. (2007). Identification of novel Wilms' tumor suppressor gene target genes implicated in kidney development. *J. Biol. Chem.* **282**, 16278-16287.
Kim, K. A., Zhao, J., Andarmani, S., Kakitani, M., Oshima, T., Binnerts, M. E., Abo, A., Tomizuka, K. and Funk, W. D. (2006). R-Spondin proteins: a novel link to β-catenin activation. *Cell Cycle* **5**, 23-26.
Klassen, M. P. and Shen, K. (2007). A Wnt gradient controls subcellular distribution of synapses through a Frizzled receptor in *C. elegans*. *Cell* In press.
Kramps, T., Peter, O., Brunner, E., Nellen, D., Froesch, B., Chatterjee, S., Murone, M., Zullig, S. and Basler, K. (2002). Wnt/wingless signaling requires BCL9/legless-mediated recruitment of pygopus to the nuclear β-catenin-TCF complex. *Cell* **109**, 47-60.
Kubo, F., Takeichi, M. and Nakagawa, S. (2005). Wnt2b inhibits differentiation of retinal progenitor cells in the absence of Notch activity by downregulating the expression of proneural genes. *Development* **132**, 2759-2770.
Larabell, C. A., Torres, M., Rowning, B. A., Yost, C., Miller, J. R., Wu, M., Kimelman, D. and Moon, R. T. (1997). Establishment of the dorso-ventral axis in *Xenopus* embryos is presaged by early asymmetries in β-catenin that are modulated by the Wnt signaling pathway. *J. Cell Biol.* **136**, 1123-1136.
Li, B., Rheaume, C., Teng, A., Bilanchone, V., Munguia, J. E., Hu, M., Jessen, S., Piccolo, S., Waterman, M. L. and Dai, X. (2007). Developmental phenotypes and reduced Wnt signaling in mice deficient for *pygopus 2*. *Genesis* **45**, 318-325.
Lin, R., Hill, R. J. and Priess, J. R. (1998). POP-1 and anterior-posterior fate decisions in *C. elegans* embryos. *Cell* **92**, 229-239.
Lin, X. (2004). Functions of heparan sulfate proteoglycans in cell signaling during development. *Development* **131**, 6009-6021.
Liu, P., Wakamiya, M., Shea, M. J., Albrecht, U., Behringer, R. R. and Bradley, A. (1999). Requirement for *Wnt3* in vertebrate axis formation. *Nat. Genet.* **22**, 361-365.
Logan, C. Y., Miller, J. R., Ferkowicz, M. J. and McClay, D. R. (1999). Nuclear β-catenin is required to specify vegetal cell fates in the sea urchin embryo. *Development* **126**, 345-357.
Lyuksyutova, A. I., Lu, C. C., Milanesio, N., King, L. A., Guo, N., Wang, Y., Nathans, J., Tessier-Lavigne, M. and Zou, Y. (2003). Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling. *Science* **302**, 1984-1988.
Ma, H., Nguyen, C., Lee, K. S. and Kahn, M. (2005). Differential roles for the coactivators CBP and p300 on TCF/β-catenin-mediated survivin gene expression. *Oncogene* **24**, 3619-3631.
Major, M. B., Camp, N. D., Berndt, J. D., Yi, X., Goldenberg, S. J., Hubbert, C., Biechele, T. L., Gingras, A. C., Zheng, N., Maccoss, M. J. et al. (2007). Wilms tumor suppressor WTX negatively regulates WNT/β-catenin signaling. *Science* **316**, 1043-1046.
Mao, B., Wu, W., Davidson, G., Marhold, J., Li, M., Mechler, B. M., Delius, H., Hoppe, D., Stannek, P., Walter, C. et al. (2002). Kremen proteins are Dickkopf receptors that regulate Wnt/β-catenin signalling. *Nature* **417**, 664-667.
Mao, J., Wang, J., Liu, B., Pan, W., Farr, G. H., 3rd, Flynn, C., Yuan, H., Takada, S., Kimelman, D., Li, L. et al. (2001). Low-density lipoprotein receptor-related protein-5 binds to Axin and regulates the canonical Wnt signaling pathway. *Mol. Cell* **7**, 801-809.
Marszalek, J. R., Ruiz-Lozano, P., Roberts, E., Chien, K. R. and Goldstein, L. S. (1999). Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc. Natl. Acad. Sci. USA* **96**, 5043-5048.
Merrill, B. J., Pasolli, H. A., Polak, L., Rendl, M., Garcia-Garcia, M. J., Anderson, K. V. and Fuchs, E. (2004). Tcf3: a transcriptional regulator of axis induction in the early embryo. *Development* **131**, 263-274.
Mikels, A. J. and Nusse, R. (2006). Purified Wnt5a protein activates or inhibits β-catenin-TCF signaling depending on receptor context. *PLoS Biol.* **4**, e115.
Miyabayashi, T., Teo, J. L., Yamamoto, M., McMillan, M., Nguyen, C. and Kahn, M. (2007). Wnt/β-catenin/CBP signaling maintains long-term murine embryonic stem cell pluripotency. *Proc. Natl. Acad. Sci. USA* **104**, 5668-5673.
Mizumoto, K. and Sawa, H. (2007). Cortical β-catenin and APC regulate asymmetric nuclear β-catenin localization during asymmetric cell division in *C. elegans*. *Dev. Cell* **12**, 287-299.
Nam, J. S., Turcotte, T. J., Smith, P. F., Choi, S. and Yoon, J. K. (2006). Mouse cristin/R-spondin family proteins are novel ligands for the Frizzled 8 and LRP6 receptors and activate β-catenin-dependent gene expression. *J. Biol. Chem.* **281**, 13247-13257.
Nelson, W. J. and Nusse, R. (2004). Convergence of Wnt, β-catenin, and cadherin pathways. *Science* **303**, 1483-1487.
Nishita, M., Yoo, S. K., Nomachi, A., Kani, S., Sougawa, N., Ohta, Y., Takada, S., Kikuchi, A. and Minami, Y. (2006). Filopodia formation mediated by receptor tyrosine kinase Ror2 is required for Wnt5a-induced cell migration. *J. Cell Biol.* **175**, 555-562.
North, T. E., Goessling, W., Walkley, C. R., Lengerke, C., Kopani, K. R., Lord, A. M., Weber, G. J., Bowman, T. V., Jang, I. H., Grosser, T. et al. (2007). Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature* **447**, 1007-1011.

Nusse, R. (2005). Wnt signaling in disease and in development. *Cell Res.* **15**, 28-32.
Oishi, I., Kawakami, Y., Raya, A., Callol-Massot, C. and Izpisua Belmonte, J. C. (2006). Regulation of primary cilia formation and left-right patterning in zebrafish by a noncanonical Wnt signaling mediator, *duboraya*. *Nat. Genet.* **38**, 1316-1322.
Pan, C. L., Howell, J. E., Clark, S. G., Hilliard, M., Cordes, S., Bargmann, C. I. and Garriga, G. (2006). Multiple Wnts and frizzled receptors regulate anteriorly directed cell and growth cone migrations in Caenorhabditis elegans. *Dev. Cell* **10**, 367-377.
Panakova, D., Sprong, H., Marois, E., Thiele, C. and Eaton, S. (2005). Lipoprotein particles are required for Hedgehog and Wingless signalling. *Nature* **435**, 58-65.
Parish, C. L. and Arenas, E. (2007). Stem-cell-based strategies for the treatment of Parkinson's disease. *Neurodegener. Dis.* **4**, 339-347.
Park, T. J., Haigo, S. L. and Wallingford, J. B. (2006). Ciliogenesis defects in embryos lacking *inturned* or *fuzzy* function are associated with failure of planar cell polarity and Hedgehog signaling. *Nat. Genet.* **38**, 303-311.
Parker, D. S., Jemison, J. and Cadigan, K. M. (2002). Pygopus, a nuclear PHD-finger protein required for Wingless signaling in *Drosophila*. *Development* **129**, 2565-2576.
Pereira, L., Yi, F. and Merrill, B. J. (2006). Repression of *Nanog* gene transcription by Tcf3 limits embryonic stem cell self-renewal. *Mol. Cell. Biol.* **26**, 7479-7491.
Phillips, B. T., Kidd, A. R., 3rd, King, R., Hardin, J. and Kimble, J. (2007). Reciprocal asymmetry of SYS-1/β-catenin and POP-1/TCF controls asymmetric divisions in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **104**, 3231-3236.
Prasad, B. C. and Clark, S. G. (2006). Wnt signaling establishes anteroposterior neuronal polarity and requires retromer in *C. elegans*. *Development* **133**, 1757-1766.
Rivera, M. N. and Haber, D. A. (2005). Wilms' tumour: connecting tumorigenesis and organ development in the kidney. *Nat. Rev. Cancer* **5**, 699-712.
Rivera, M. N., Kim, W. J., Wells, J., Driscoll, D. R., Brannigan, B. W., Han, M., Kim, J. C., Feinberg, A. P., Gerald, W. L., Vargas, S. O. et al. (2007). An X chromosome gene, *WTX*, is commonly inactivated in Wilms tumor. *Science* **315**, 642-645.
Salinas, P. C. (2005). Signaling at the vertebrate synapse: new roles for embryonic morphogens? *J. Neurobiol.* **64**, 435-445.
Schambony, A. and Wedlich, D. (2007). Wnt-5A/Ror2 regulate expression of *XPAPC* through an alternative noncanonical signaling pathway. *Dev. Cell* **12**, 779-792.
Schneider, S. Q. and Bowerman, B. (2007). β-catenin asymmetries after all animal/vegetal- oriented cell divisions in *Platynereis dumerilii* embryos mediate binary cell-fate specification. *Dev. Cell* **13**, 73-86.
Schneider, S., Steinbeisser, H., Warga, R. M. and Hausen, P. (1996). B-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech. Dev.* **57**, 191-198.
Schwab, K. R., Patterson, L. T., Hartman, H. A., Song, N., Lang, R. A., Lin, X. and Potter, S. S. (2007). *Pygo1* and *Pygo2* roles in Wnt signaling in mammalian kidney development. *BMC Biol.* **5**, 15.
Simons, M., Gloy, J., Ganner, A., Bullerkotte, A., Bashkurov, M., Kronig, C., Schermer, B., Benzing, T., Cabello, O. A., Jenny, A. et al. (2005). *Inversin*, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. *Nat. Genet.* **37**, 537-543.
Song, N., Schwab, K. R., Patterson, L. T., Yamaguchi, T., Lin, X., Potter, S. S. and Lang, R. A. (2007). *pygopus 2* has a crucial, Wnt pathway-independent function in lens induction. *Development* **134**, 1873-1885.
Speese, S. D. and Budnik, V. (2007). Wnts: up-and-coming at the synapse. *Trends Neurosci.* **30**, 268-275.
Takada, S., Stark, K. L., Shea, M. J., Vassileva, G., McMahon, J. A. and McMahon, A. P. (1994). *Wnt-3a* regulates somite and tailbud formation in the mouse embryo. *Genes Dev.* **8**, 174-189.
Takemaru, K., Yamaguchi, S., Lee, Y. S., Zhang, Y., Carthew, R. W. and Moon, R. T. (2003). Chibby, a nuclear β-catenin-associated antagonist of the Wnt/Wingless pathway. *Nature* **422**, 905-909.
Tamai, K., Zeng, X., Liu, C., Zhang, X., Harada, Y., Chang, Z. and He, X. (2004). A mechanism for Wnt coreceptor activation. *Mol. Cell* **13**, 149-156.
Thompson, B., Townsley, F., Rosin-Arbesfeld, R., Musisi, H. and Bienz, M. (2002). A new nuclear component of the Wnt signalling pathway. *Nat. Cell Biol.* **4**, 367-373.
Torres, M. A., Yang-Snyder, J. A., Purcell, S. M., DeMarais, A. A., McGrew, L. L. and Moon, R. T. (1996). Activities of the Wnt-1 class of secreted signaling factors are antagonized by the Wnt-5A class and by a dominant negative cadherin in early *Xenopus* development. *J. Cell Biol.* **133**, 1123-1137.
Tzahor, E. (2007). Wnt/β-catenin signaling and cardiogenesis: timing does matter. *Dev. Cell* **13**, 10-13.
Wallingford, J. B. and Habas, R. (2005). The developmental biology of Dishevelled: an enigmatic protein governing cell fate and cell polarity. *Development* **132**, 4421-4436.
Wang, Q. T., Piotrowska, K., Ciemerych, M. A., Milenkovic, L., Scott, M. P., Davis, R. W. and Zernicka-Goetz, M. (2004). A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo. *Dev. Cell* **6**, 133-144.
Wei, Q., Yokota, C., Semenov, M. V., Doble, B., Woodgett, J. and He, X. (2007). R-spondin1 is a high affinity ligand for LRP6 and induces LRP6 phosphorylation and β-catenin signaling. *J. Biol. Chem.* **282**, 15903-15911.
Wikramanayake, A. H., Huang, L. and Klein, W. H. (1998). β-Catenin is essential for patterning the maternally specified animal-vegetal axis in the sea urchin embryo. *Proc. Natl. Acad. Sci. USA* **95**, 9343-9348.
Wikramanayake, A. H., Hong, M., Lee, P. N., Pang, K., Byrum, C. A., Bince, J. M., Xu, R. and Martindale, M. Q. (2003). An ancient role for nuclear β-catenin in the evolution of axial polarity and germ layer segregation. *Nature* **426**, 446-450.
Yoon, J. K. and Wold, B. (2000). The bHLH regulator pMesogenin1 is required for maturation and segmentation of paraxial mesoderm. *Genes Dev.* **14**, 3204-3214.
Zhang, X., Zhu, J., Yang, G. Y., Wang, Q. J., Qian, L., Chen, Y. M., Chen, F., Tao, Y., Hu, H. S., Wang, T. et al. (2007). Dishevelled promotes axon differentiation by regulating atypical protein kinase C. *Nat. Cell Biol.* **9**, 743-754.
Zou, Y. (2004). Wnt signaling in axon guidance. *Trends Neurosci.* **27**, 528-532.