

A WNTer wonderland in Snowbird

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The Keystone Symposium on 'Wnt and β -catenin signaling in development and disease' was held recently in Snowbird, UT, USA. Organized by Mariann Bienz and Hans Clevers, this meeting covered a wide range of topics, including Wnt protein biogenesis, Wnt receptors and signaling pathways, β -catenin/Tcf complexes and gene expression, Wnt signaling in development, cancer, stem cell biology and regeneration, and therapeutics that target the Wnt/ β -catenin pathway.

Introduction

The Wnt family of secreted signaling molecules regulates many aspects of animal development and tissue homeostasis, and abnormal Wnt signaling has been associated with human diseases, such as cancer and osteoporosis (Logan and Nusse, 2004; Clevers, 2006). The study of Wnt signaling has transcended a vast landscape of biomedical research, from the basic mechanism of embryogenesis to stem cell biology and carcinogenesis, and therapeutics and drug discovery.

Wnt proteins: modification, secretion and gradient formation

Wnt proteins exhibit both short- and long-range signaling capabilities and are thought to function as morphogens (Zecca et al., 1996). The molecular basis of these properties is not well understood, but is probably related to Wnt protein biogenesis. Wnt proteins are poorly secreted and notoriously difficult to purify. Roel Nusse (Stanford University, Stanford, CA, USA) described the successful purification of several Wnt proteins, including *Drosophila* Wingless (Wg), and murine Wnt3a and Wnt5a. In addition to facilitating the analysis of early signaling responses, the purification of Wnt3a revealed that Wnt proteins are palmitoylated on a conserved cysteine residue (Cys77 in Wnt3a) (Willert et al., 2003). This lipid modification, which is crucial for Wnt function, was discussed by Shinji Takada (Okazaki Institute for Integrative Bioscience, Okazaki, Japan) and Laura Burrus (San Francisco State University, San Francisco, CA, USA). Wg palmitoylation and secretion depend on Porcupine, a multi-pass transmembrane (TM) protein and putative O-acyltransferase that resides in the endoplasmic reticulum (Kadowaki et al., 1996; van den Heuvel et al., 1993; Zhai et al., 2004). Takada and Burrus each showed that Wnt3a palmitoylation and secretion require the mammalian Porcupine ortholog. They also suggested that in addition to Cys77, another Wnt3a palmitoylation site (or sites) is/are likely to exist and to be important for Wnt3a activity, secretion and gradient formation in the developing *Xenopus* blastula and chick neural tube.

Konrad Basler (University of Zurich, Zurich, Switzerland) and Hendrik Korswagen (Netherlands Institute of Developmental Biology, Utrecht, The Netherlands) described two new components involved in Wnt secretion. Basler identified the *wntless* (*wls*) gene in a suppressor screen for a Wg gain-of-function phenotype (Banziger et al., 2006) (see also Bartscherer et al., 2006). The *wls* gene encodes a seven-pass TM protein that is conserved from *C. elegans* to humans. The *C. elegans* *wls* ortholog, *mom-3*, is required for *mom-2*/Wnt function (Thorpe et al., 1997). Wg/Wnt proteins are not secreted in the *Drosophila* *wls* mutant or from mammalian cells deprived of Wls, indicating that Wls is essential for Wnt protein maturation/secretion (Fig. 1A). Korswagen identified *vps-35* in an RNAi screen performed in *C. elegans* to identify components of the *egl-20*/Wnt pathway (Coudreuse et al., 2006) (also see Prasad and Clark, 2006). VPS-35 is a subunit of the 'retromer' complex, which is required for intracellular protein trafficking in yeast. Similar to Wls (Banziger et al., 2006), expression of *vps-35* in EGL-20/Wnt-producing cells, but not in responding cells, rescues mutant phenotypes. However, VPS-35 does not appear to affect Wnt secretion per se, but rather disrupts long-range, but not short-range, EGL-20/Wnt signaling. VPS-35 and the retromer complex may guide Wnt to a specific secretory pathway for long-range gradient formation (Coudreuse et al., 2006) (Fig. 1A). Like Porcupine, Wls and VPS-35 seem to be dedicated specifically to Wnt biogenesis.

Wnt receptors and downstream components

Nusse showed that purified Wnt5a can activate canonical β -catenin signaling in the presence of Frizzled4 (Fz4), one of the Fz Wnt receptors, and a co-receptor, LDL receptor-related protein 5 (Lrp5). But Wnt5a can also antagonize Wnt3a signaling via a putative tyrosine kinase receptor Ror2, downstream of β -catenin stabilization (Mikels and Nusse, 2006) (Fig. 1B,C). Nusse argued that the receptor complement determines Wnt signaling output and that Wnt proteins should not be classified as being intrinsically canonical or non-canonical. Xi He (Children's Hospital/Harvard Medical School, Boston, MA, USA), Christof Niehrs (German Cancer Research Center, Heidelberg, Germany), Akira Kikuchi (Hiroshima University, Hiroshima, Japan), Paul Polakis (Genentech, South San Francisco, CA, USA) and Anna Bafico (Mount Sinai School of Medicine, New York, NY, USA) discussed the co-receptor Lrp6. Lrp6 is essential for β -catenin pathway activation (He et al., 2004) and is activated via Wnt-induced phosphorylation at its PPPSPxS motifs (Tamai et al., 2004), which are docking sites for Axin (Mao et al., 2001) (see Fig. 1C). He demonstrated that Gsk3 and Ck1 are sequential kinases for Lrp6 phosphorylation and activation, and that Wnt signaling induces Gsk3 phosphorylation of PPPSP (Zeng et al., 2005). This model challenges the view that Gsk3 is solely an inhibitor of Wnt/ β -catenin signaling (via β -catenin degradation) and implies that Gsk3 intricately regulates Wnt signaling. In agreement, Niehrs identified Ck1 γ as a kinase for Lrp6, but showed that Ck1 γ phosphorylation, but not PPPSP phosphorylation, is Wnt-inducible (Davidson et al., 2005). Polakis demonstrated MAP kinase phosphorylation of the PPPSP motif in vitro. Further experimentation will clarify the Lrp6 phosphorylation issue. Kikuchi discussed Lrp6 interaction with Caveolin, a structural protein of caveolae (vesicular invaginations of the plasma membrane). Wnt3a induces Lrp6 internalization and Lrp6-Caveolin association, and siRNA depletion of Caveolin (but not of Clathrin) or a dominant-negative Dynamin mutant inhibits

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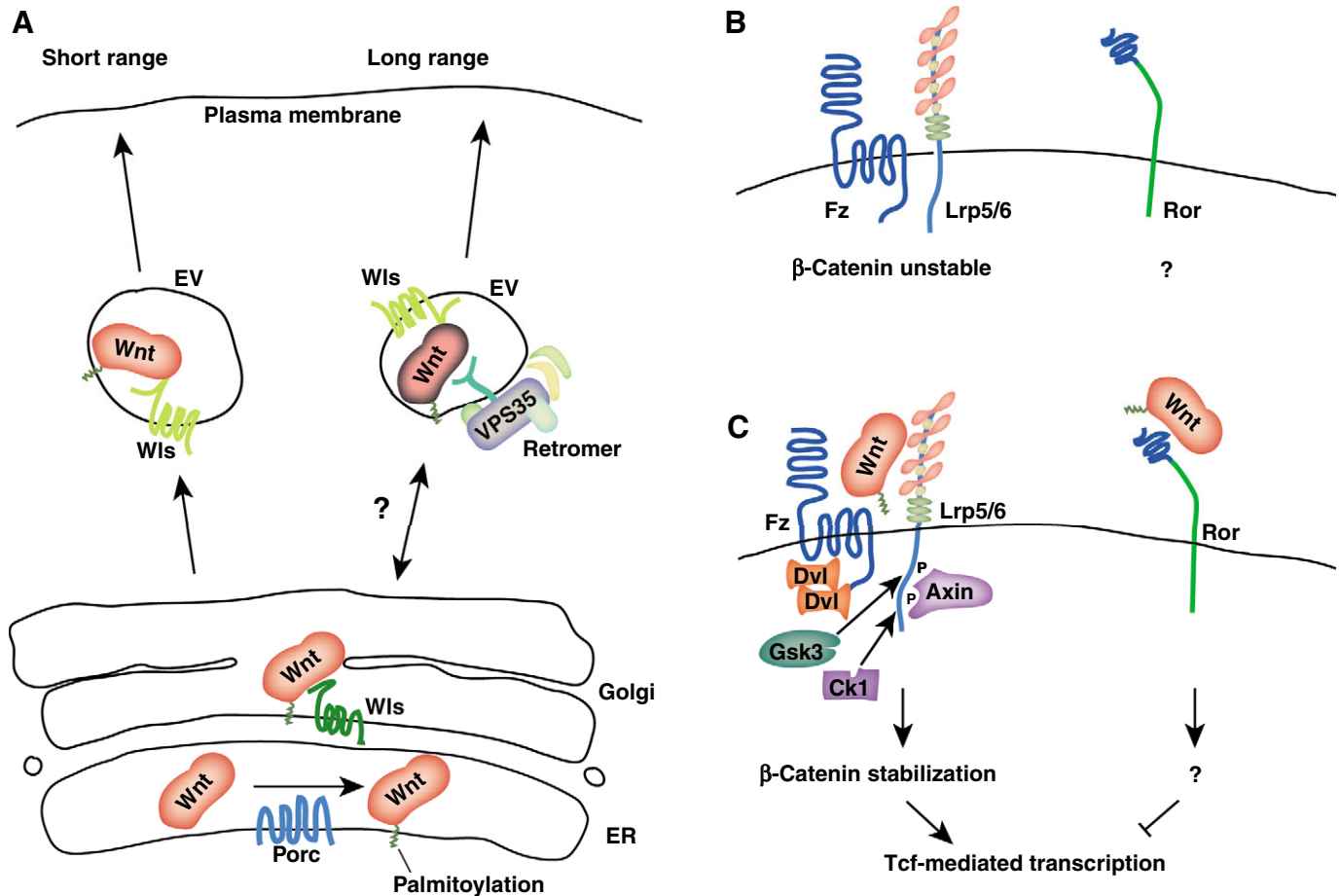


Fig. 1. Wnt biogenesis and interaction with receptors. (A) Wnt biogenesis for short- and long-range signaling. Wnt palmitoylation occurs within the endoplasmic reticulum (ER) and requires Porcupine (Porc). Wntless (Wls) probably acts in the Golgi apparatus and exocytic vesicles (EV), and is required for Wnt to reach the cell surface for secretion (Banziger et al., 2006; Bartscherer et al., 2006). VPS-35 and the retromer complex appears to be required only for Wnt (EGL-20) destined for long-range signaling (Coudreuse et al., 2006; Prasad and Clark, 2006). (B) In the absence of Wnt, β -catenin is unstable. (C) The association of Wnt with the Fz-Lrp5/6 co-receptor complex stabilizes β -catenin, via the sequential phosphorylation (P) of the Lrp5/6 cytoplasmic domain, likely by Gsk3 and Ck1 γ (Davidson et al., 2005; Zeng et al., 2005), to activate the canonical pathway. Lrp5/6 phosphorylation recruits the scaffolding protein Axin (Mao et al., 2001; Tamai et al., 2004). Dishevelled (Dvl) may act via the recruitment or inhibition of the Axin complex. Wnt5a can bind Ror2, and antagonize Tcf/ β -catenin transcription downstream of β -catenin stabilization (Mikels and Nusse, 2006).

Lrp6 internalization and Wnt signaling. Kikuchi suggested that Caveolin-mediated Lrp6 endocytosis is important for Wnt/ β -catenin signaling. Bafico showed that Lrp6 mediates an autocrine Wnt signal in some breast cancer cell lines (Bafico et al., 2004), some of which exhibit Lrp6 overexpression. She also observed in an ovarian cancer line the expression of a Lrp6 variant that is Wnt-responsive but is resistant to inhibition by Dkk1 (a Lrp6 antagonist) (He et al., 2004).

Dishevelled (Dsh/Dvl) is required for multiple Wnt/Fz pathways but its function remains enigmatic (Wallingford and Habas, 2005). Mariann Bienz (MRC Laboratory of Molecular Biology, Cambridge, UK) demonstrated that the cytoplasmic Dvl2 'dots' are not vesicles but rather dynamic Dvl protein multimers (Schwarz-Romond et al., 2005) (also see Smalley et al., 2005), which forms via the DIX domain of the protein. She showed that recombinant Dvl2 DIX domain polymerizes gradually and reversibly in vitro. As Dvl2 can recruit Axin into its assemblies, Bienz proposed that DIX domain-mediated Dvl polymerization promotes the efficient recruitment of Axin, resulting in β -catenin signaling.

β -Catenin and Tcf/Lef complex

The 'simple' model that Wnt signaling stabilizes β -catenin to lead to β -catenin-Tcf/Lef complex formation, which activates downstream gene expression, is widely cited. In the absence of Wnt, Tcf/Lef associates with co-repressors, including Groucho/Tle to suppress gene expression (Stadali et al., 2006) (Fig. 2A). Bill Weis (Stanford University, Stanford, CA, USA) demonstrated, by using recombinant proteins, that β -catenin-Lef1 and Groucho-Lef1 complexes are mutually exclusive, owing to β -catenin binding to a previously unrecognized low-affinity site within Lef1 that overlaps with the Groucho-binding domain (Daniels and Weis, 2005). Wenqing Xu (University of Washington, Seattle, WA, USA) discussed the crystal structure of full-length β -catenin, revealing additional structural components that may provide new interfaces for known and novel β -catenin partners. A crystal structure that contained fragments of Tcf, β -catenin and Bcl9 (a co-activator) (Fig. 2B) also revealed that the β -catenin-Bcl9 interface is distinct from most other β -catenin-interacting proteins, highlighting a potential target for specific therapeutic intervention.

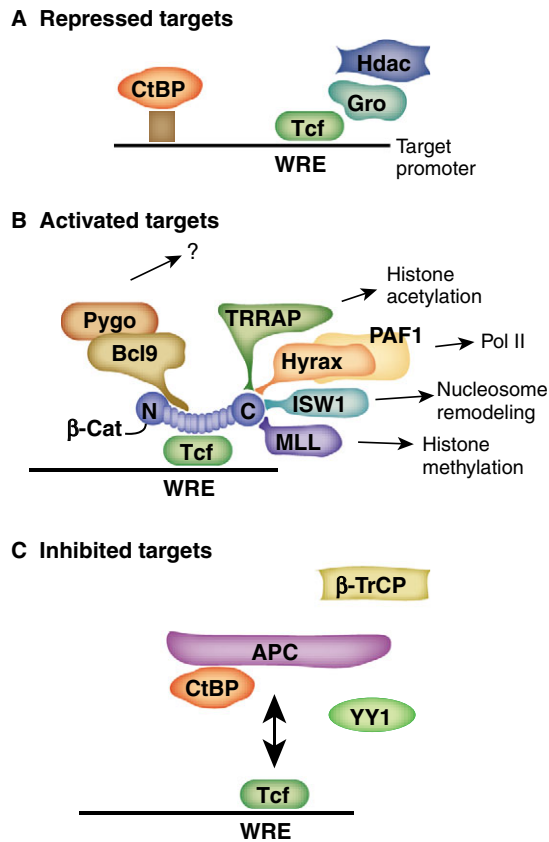


Fig. 2. Nuclear Tcf/β-catenin complexes. (A) In the absence of Wnt stimulation, Tcf binds to the WRE (Wnt responsive element) but recruits Groucho-HDAC to repress Wnt responsive genes. CtBP is involved in this repression but acts in parallel to Tcf (Fang et al., 2006). (B) On Wnt stimulation, stabilized β-catenin complexes with Tcf and recruits multiple co-activator complexes for transcriptional activation (Mosimann et al., 2006; Sierra et al., 2006). (C) When wild-type APC is expressed in cancer cells that harbor a mutant APC, wild-type APC directly inhibits *Myc* expression (Sierra et al., 2006) by recruiting CtBP to the *Myc* promoter (Sierra et al., 2006). A transcriptional repressor, YY1, and the E3 ubiquitin ligase subunit β-Trcp, which is required for β-catenin degradation, are also recruited to the *Myc* promoter (Sierra et al., 2006). The mechanism by which many of these events occur remains unknown. APC may also act together with CtBP to remove β-catenin from Tcf (Hamada and Bienz, 2004).

Basler and Kathy Jones (Salk Institute, La Jolla, CA, USA) described several novel β-catenin co-activators. Basler identified Hyrax/Parafibromin, which is required for β-catenin-dependent transcription in *Drosophila* and human cells and is a component of the PAF1 (polymerase associated factor 1) complex (Mosimann et al., 2006). Hyrax/Parafibromin and Bcl9/Legless-pygopus (Stadali et al., 2006) bind β-catenin C- and N-terminal transactivation domains (CTD and NTD) (Fig. 2B), respectively, and act in parallel or sequentially in β-catenin-mediated transcription. Jones identified the TRRAP/TIP60 histone acetylation complex, the ISW1 nucleosome remodeling complex and MLL1/MLL2 histone methylation complexes as binding partners of β-catenin CTD (Fig. 2B). β-catenin-Lef1 recruits these complexes, and Bcl9-pygopus to the *Myc* (previously known as *c-Myc*) gene, and promotes histone H4 acetylation and histone H3K4 (lysine 4) trimethylation (Sierra et al., 2006). The APC tumor suppressor, the *adenomatous polyposis*

coli gene product, antagonizes *Myc* expression, but (surprisingly) by acting directly on the *Myc* gene together with co-repressors, including CtBP (Hamada and Bienz, 2005) (Fig. 2C). Apc and CtBP are subsequently replaced by other co-repressors, such as Groucho and Hdac1 (histone deacetylase 1) (Sierra et al., 2006). Jones proposed that Apc has a direct role in promoting the exchange of co-activators with co-repressors on β-catenin-Tcf/Lef target genes. Ken Cadigan (University of Michigan, Ann Arbor, MI, USA) examined the role of CtBP in the Wg pathway in fly cells, reporting that CtBP directly represses the *naked cuticle* gene in parallel to Tcf-Groucho (Fang et al., 2006) (Fig. 2A). This mechanism is distinct from previous reports (Hamada and Bienz, 2005; Sierra et al., 2006), in that CtBP repression occurs in the absence of β-catenin/Armadillo. Cadigan also provided data that CtBP plays a positive role in Wg signaling, being recruited by β-catenin/Armadillo to some Wg targets (Fang et al., 2006).

Stefan Hoppler (University of Aberdeen, UK) and Marian Waterman (University of California, Irvine, CA, USA) discussed the complexity of the vertebrate Tcf/Lef family. Hoppler showed that both Tcf1 and Tcf3 are required for *Xenopus* mesoderm formation, but act in transcriptional activation and repression, respectively, whereas both Tcf1 and Lef1 are required for ventrolateral patterning by acting as transcription activators (Liu et al., 2005). Multiple isoforms can be derived from alternative promoters and/or by alternative splicing of each Tcf/Lef gene. Waterman reported that Tcf1E and Tcf4E contain a novel DNA-binding domain in the 'E-tail', which binds double-stranded DNA (but with little sequence specificity) and seems to allow Tcf1E and Tcf4E to regulate a larger set of genes. In colon tissues, Tcf1E predominantly acts as a repressor because it lacks the β-catenin-binding domain, and antagonizes Tcf4E transactivation. In colon cancer cells, however, Tcf1E becomes cytoplasmic, leaving Tcf4E unopposed in the nucleus and presumably enabling higher Tcf4E-β-catenin transactivation.

Development

Wnt signaling induces vertebrate limb bud mesenchyme to proliferate (Capdevila and Izpisua Belmonte, 2001). Wnt3a-induced mesenchymal proliferation, reported Nusse, requires the induction and function of *N-Myc* (*Mycn* – Mouse Genome Informatics), as proliferation is prevented in *N-Myc* knockout mice. Wnt3a simultaneously blocks differentiation (chondrogenesis), which, interestingly, is *N-Myc*-independent and is probably mediated via *Sox9* inhibition. Christine Perret (Institut Cochin, Paris, France) reported a key role for Wnt signaling in liver patterning and function from studies of mice with acute *Apc* deletion or forced *Dkk1* expression. Differential gene expression along the perivenous to periportal axis depends on the activation and suppression of β-catenin signaling, respectively, which correlates with the complementary expression of active β-catenin in the perivenous region and of *Apc* in the periportal region (Benhamouche et al., 2006). By conditionally deleting or activating β-catenin, Walter Birchmeier (Max Delbrück Center for Molecular Medicine, Berlin, Germany) explored the function of β-catenin and BMP signaling in patterning the dorsal neural tube, particularly in the allocation of dl2-dl6 neuronal fates. He also demonstrated two 'opposing' roles of β-catenin signaling in lens induction and in the restriction of the lens field.

Pygopus and Legless/Bcl9 are essential partners/co-activators of Wg/β-catenin signaling in flies (Belenkaya et al., 2002; Kramps et al., 2002; Parker et al., 2002; Thompson et al., 2002). Xing Dai (University of California, Irvine, CA, USA) knocked out the gene encoding pygopus 2 in mice and found phenotypes consistent with

decreased, but not abolished, Wnt signaling in mammary glands, hair follicles, and the lung. Given the restricted expression pattern of the other homolog, *pygopus 1* (Li et al., 2004), the results suggest that *pygopus* is not essential for Wnt signaling but facilitates signaling amplification. Birchmeier and Michel Aguet (Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland) each made *pygopus 1* and 2 double knockout mice. Each found failure of lens development associated with loss of *Pax6* expression. Aguet also reported the incomplete migration of cardiac neural crest and failed aortic arch formation, associated with the decreased expression of specific Wnt target genes, in addition to a general attenuation (but not elimination) of a Tcf reporter expression (BAT-Gal) in these double mutants. Strikingly, in all three laboratories, the mutants fail to recapitulate the full spectrum of Wnt/ β -catenin signaling defects. Aguet also deleted both *Bcl9* and *Bcl9-2*, and *pygopus 1* and 2, in the intestine, which requires Wnt/ β -catenin signaling for development and homeostasis (Gregorieff and Clevers, 2005). Surprisingly, he observed no overt gastrointestinal abnormality. However, intestinal regeneration after injury did occur in the *pygopus 1* and 2 double mutant, but not in the *Bcl9/Bcl9-2* double mutant intestines. There was a spirited debate about whether *pygopus 1* and 2, and *Bcl9* and 9-2 are only required for a subset of Wnt signaling in mice or whether the described deletions generate null alleles. Perhaps multiple co-activator complexes (Sierra et al., 2006) can lead to β -catenin transactivation in different contexts.

Jeff Axelrod (Stanford University, Stanford, CA, USA) was one of several speakers that discussed non-canonical Wnt or Fz signaling. He described a local feedback loop model for planar cell polarity (PCP) signaling that depends on the mutual recruitment of Fz and Van Gogh/Strabismus to adjacent membranes of neighboring cells, and that serves to align their polarities in a domino fashion in fly wing epithelium. By combining mathematical modeling with experimentation (Amonlirdviman et al., 2005), he demonstrated how some alleles of Fz affect the polarity of neighboring wild-type cells. He also showed that PCP propagation is sensitive to cell geometry. Ping Chen (Emory University, Atlanta, GA, USA) described the role of PCP signaling in stereocilia orientation and cellular intercalation in the organ of Corti. She showed that, analogous to the *Drosophila* wing, *Vangl2* (a Van Gogh homolog) and *Dvl2* exhibit polarized medial and lateral localization, respectively, in a PCP signaling-dependent manner (Wang et al., 2005; Wang et al., 2006). *Wnt5a* and *Frzb*, a Wnt antagonist, are candidates for establishing PCP. *Wnt5a*^{-/-} mice show cochlear duct malformation, which is consistent with *Wnt5a* functioning in cochlear convergent extension, but no apparent stereocilia orientation defect.

Sergei Sokol (Mount Sinai School of Medicine, New York, NY, USA) discussed the interaction of Dsh with Lgl (lethal giant larvae), a protein important for epithelial apical basal polarity. In *Xenopus* and *Drosophila* embryos, loss of Dsh function causes defective epithelial polarity and Lgl to mislocalize from its normal basolateral cortex. Fz8 (but not Fz7) overexpression results in Lgl mislocalization, suggesting a mechanism by which Fz signaling regulates apical basal polarity (Dollar et al., 2005). Lgl depletion in *Xenopus* embryos causes defective gastrulation movements similar to those seen in embryos with abnormal Fz/Dsh PCP signaling, indicating that Fz/Dsh regulation of PCP and apical basal polarity may be coupled. Katherine Harris (University of California, Berkeley, CA, USA) reported that multiple Wnt pathways regulate fly salivary gland migration mediated by the repellents *Wnt5* and *Wnt4*. Mutations in the *Wnt5* receptor gene *derailed* (Yoshikawa et al., 2003; He, 2005), and in *fz*, *fz2*, *dsh* and *tcf* all lead to abnormal

salivary gland migration. Harris proposed that *Wnt5*-Derailed and *Wnt4*-Fz/Fz2 activate β -catenin-independent and -dependent pathways, respectively, to coordinate the repulsive response during migration.

Cancer

Marc van de Wetering from Hans Clevers' group (Netherlands Institute of Developmental Biology, Utrecht, The Netherlands) described continuing effects to dissect the Tcf/ β -catenin regulatory network in colon cancers. Their analyses indicate that Wnt/ β -catenin signaling controls three aspects of intestinal homeostasis: stem cell renewal, proliferation of transient amplifying progenitors, and Paneth cell maturation. Mark Taketo (Kyoto University, Kyoto, Japan) showed that activated Tcf/ β -catenin signaling, via APC loss or activated β -catenin, induces chromosomal instability in mouse intestinal polyps and embryonic stem cells. Alan Clarke (Cardiff University, Cardiff, UK) reported the events that follow acute *Apc* loss in the mouse intestine, which include increased proliferation, failure of differentiation and migration, and aberrant apoptosis (Sansom et al., 2004). Expression profiling revealed that many 'known' Tcf- β -catenin targets are not induced until adenoma development, and are therefore indirect targets, such as cyclin D1 (Sansom et al., 2005). Clarke discussed two novel targets, Sparc (an extracellular protein) and Mbd2 (a methylated CpG binding protein); the absence of each suppresses tumorigenesis induced by *Apc* loss (e.g. Sansom et al., 2003) (whereas the absence of Cyclin D1, Tcf1, or p53 does not). Using the same paradigm, Owen Sansom (Beatson Institute of Cancer Research, Glasgow, UK) demonstrated that tumors induced by *Apc* loss are completely inhibited when Myc is removed; normal crypt architecture is restored despite high levels of nuclear β -catenin. Interestingly, over half of known β -catenin target genes were not induced in the absence of Myc. Thus Myc, like Sparc and Mbd2, is essential for tumorigenesis caused by *Apc* loss; they may therefore represent attractive therapeutic targets. Mark Peifer (University of North Carolina, Chapel Hill, NC, USA), in collaboration with Brooke McCartney (Carnegie Mellon University, Pittsburgh, PA, USA), investigated an allelic series of *Drosophila APC1* and *APC2* mutations, including a protein null. In *APC1* and *APC2* double-null embryos, several cellular processes in which APC functions have been implicated are generally normal, including mitotic spindle formation, epithelial cell division and cadherin-based cell junction formation (McCartney et al., 2006). These results argue that many phenotypes caused by existing alleles and, by analogy, by APC truncations in cancer, probably reflect dominant-negative (neomorphic) functions. Their results also indicate that the APC truncations are strong, but not null, with regard to Wg/ β -catenin signaling (McCartney et al., 2006).

David Jones (University of Utah, Salt Lake City, UT, USA) explored the relationship between APC and retinoic acid (RA) in colon tissue and in a zebrafish model (Nadauld et al., 2004; Nadauld et al., 2005). He showed that APC induces RA biosynthesis through a β -catenin-independent mechanism and blocks its catabolism through a β -catenin-dependent mechanism. RA is in turn required for colonocyte differentiation and suppresses Cox2 (cyclooxygenase 2) expression. Raymond DuBois (Vanderbilt-Ingram Cancer Center, Nashville, TN, USA) also discussed Cox2, as it is upregulated in invasive colon cancers and lies downstream of many tumor promoters, including Wnt/ β -catenin signaling. Recent evidence suggests that Cox2-dependent PGE2 (prostaglandin E2) production feeds back to augment β -catenin stabilization through the EP2 G-protein coupled receptor (GPCR) (Castellone et al., 2005). DuBois presented yet another Cox2 pathway, in which PGE2 binds the EP4

GPCR and activates a β -arrestin/Src/Egf receptor pathway, leading to the activation of the Akt kinase and to tumor metastasis (Buchanan et al., 2006).

Stem cell and regeneration

Elaine Fuchs (Rockefeller University, New York, NY, USA) and Tannishtha Reya (Duke University, Durham, NC, USA) focused on the role of Wnt/ β -catenin signaling in stem cell regulation. Fuchs discussed adult hair follicle stem cells (SCs), which reside in the niche (bulge) (Alonso and Fuchs, 2003) and require β -catenin for their maintenance (Lowry et al., 2005). Tcf3 maintains SCs in a growth- and differentiation-inhibited state. During normal hair cycle or injury, SCs are activated by β -catenin signaling to proliferate and exit the niche. Lef1- β -catenin signaling is then required to direct these activated SCs down the hair cell lineage (Lowry et al., 2005). Thus, the transition from Tcf3 suppression to Lef1- β -catenin activation governs stem cell maintenance versus activation and differentiation. Reya examined the role of β -catenin in hematopoietic stem cells (HSCs) (Reya and Clevers, 2005). She reported that conditional loss of β -catenin in HSCs allows a normal hematopoietic compartment to develop but leads to a significant reduction in the ability of HSCs to renew and maintain hematopoiesis *in vivo* following transplantation. She also found that β -catenin loss in a Bcr-Abl-driven leukemia mouse model slowed disease progression and significantly reduced the incidence of chronic myelogenous leukemia, a neoplasm that arises in stem cells. Therefore, β -catenin is required for long term self-renewal of normal and malignant stem cells in the hematopoietic system.

Several talks addressed Wnt in regeneration. Randall Moon (University of Washington, Seattle, WA, USA) showed that Wnt genes and Tcf/ β -catenin signaling are induced in all regenerating tissues examined, from zebrafish, frog to mouse. Wnt/ β -catenin signaling is required, via promoting proliferation, for fish fin regeneration (Fig. 3), as forced expression of Dkk1 or blocking Tcf prevents regeneration. Nusse reported similar observations in the study of lung epithelial regeneration. Moon showed that Wnt5a is also induced during, but antagonizes, fin regeneration, and probably by counterbalancing β -catenin signaling. Whether Wnt/ β -catenin signaling regulates rare stem cells or promotes dedifferentiation at injury sites remains unanswered. Juan Carlos Izpisua-Belmonte (Salk Institute, La Jolla, CA, USA) considered the difference between animals that retain the ability to regenerate a limb after birth, such as urodele amphibians and teleost fish, versus other animals, and hypothesized that the key difference may be the absence of the apical ectodermal ridge (AER), a stratified epithelium and a source of many signals, in regeneration-competent limbs. Indeed, he observed regeneration in the chick limb after removing the AER and expressing β -catenin in the underlying mesenchyme.

Cnidarians are remarkable models for regeneration studies (Holstein et al., 2003). Cnidarian genomes have recently been shown to encode an unexpected diversity of Wnt genes, possessing at least 11 of the 12 Wnt subfamilies carried by bilaterians (Kusserow et al., 2005). In *Nematostella vectensis*, a basal cnidarian, Thomas Holstein (Heidelberg University) showed that eight Wnt genes have serial, overlapping expression in rings around the blastopore, and that Dkk proteins (Wnt antagonists) are expressed in an inverse gradient (Guder et al., 2006) (Fig. 3). Pharmacological intervention indicates a role for Wnt signaling in head regeneration. In Hydra, there is also evidence of β -catenin-dependent signaling during head induction, and of β -catenin-independent signaling during tentacle and bud morphogenesis.

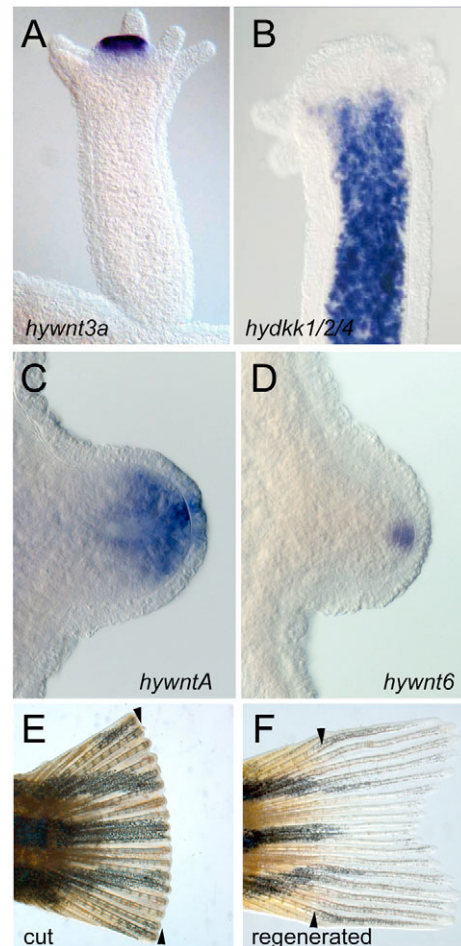


Fig. 3. Wnt in regeneration. (A,B) Inverse gradients of Wnt and Dkk proteins in Hydra pattern formation during budding and regeneration. (C,D) Overlapping ring-like expression domains of hywntA (C) and hywnt6 (D) during Hydra bud formation (H. Bode and T. Holstein, unpublished). (E,F) Zebrafish tail fin regeneration, which requires Wnt/ β -catenin signaling (R. Moon, personal communication). Image courtesy of Christi Stoick, University of Washington, Seattle, WA, USA. (A) Reproduced, with permission, from Hobmayer et al. (Hobmayer et al., 2000). (B) Reproduced, with permission, from Guder et al. (Guder et al., 2006).

Therapeutic targets

In the therapeutic targets talks, Kyung-Ah Kim (Nuvelo, San Carlos, CA, USA) discussed secreted R-spondin proteins, which can induce intestinal epithelial proliferation via β -catenin signaling (Kim et al., 2005; Kazanskaya et al., 2004), but their mechanism of action and relationship with Wnt proteins remain unclear. Xiaoyan Zhang (Curis, Cambridge, MA, USA) discussed two small molecule antagonists identified in a cell-based Wnt/Tcf-reporter assay. Their targets remain to be identified, although one may act at the receptor level. Ramesh Shivdasani (Dana Farber Cancer Institute and Harvard Medical School, Boston, MA, USA) described a compound, identified from a screen performed with Novartis, that appears to specifically disrupt the Tcf4- β -catenin interaction (Lepourcelet et al., 2004). Jie Zheng (St Jude Children's Hospital, Memphis, TN, USA) described an *in silico* screen to identify an inhibitor of the Dsh PDZ domain-Fz interaction, which partially blocks Wnt signaling *in vivo* (Shan et al., 2005). He also identified a compound that binds to a domain of Lrp5 and interferes with

Dkk1-Lrp5 interaction, thereby activating the pathway. Polakis described his effort to use phage display to identify small peptides that bind Dvl and inhibit Tcf-reporter expression and showed that Wnt inhibitors such as Dkk1 and the Fz8CRD (cysteine-rich domain) can reduce tumor growth in mice.

Conclusion

Many challenges await the Wnt field. We have much to learn about Wnt proteins and their biosynthetic pathways, and Wnt-receptor interactions and signaling propagation, as well as the plethora of cytoplasmic and nuclear changes triggered by Wnt proteins. We also need a better understanding of the involvement of Wnt/ β -catenin and other Wnt pathways in embryogenesis, cancer/stem cell biology and regeneration. A WNTer wonderland is on the horizon.

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