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REVIEW

Deciphering the function of canonical Wnt signals in development and disease: conditional loss- and gain-of-function mutations of β -catenin in mice

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Wnt signaling is one of a handful of powerful signaling pathways that play crucial roles in the animal life by controlling the genetic programs of embryonic development and adult homeostasis. When disrupted, these signaling pathways cause developmental defects, or diseases, among them cancer. The gateway of the canonical Wnt pathway, which contains >100 genes, is an essential molecule called β -catenin (Armadillo in *Drosophila*). Conditional loss- and gain-of-function mutations of β -catenin in mice provided powerful tools for the functional analysis of canonical Wnt signaling in many tissues and organs. Such studies revealed roles of Wnt signaling that were previously not accessible to genetic analysis due to the early embryonic lethality of conventional β -catenin knockout mice, as well as the redundancy of Wnt ligands, receptors, and transcription factors. Analysis of conditional β -catenin loss- and gain-of-function mutant mice demonstrated that canonical Wnt signals control progenitor cell expansion and lineage decisions both in the early embryo and in many organs. Canonical Wnt signaling also plays important roles in the maintenance of various embryonic or adult stem cells, and as recent findings demonstrated, in cancer stem cell types. This has opened new opportunities to model numerous human diseases, which have been associated with deregulated Wnt signaling. Our review summarizes what has been learned from genetic studies of the Wnt pathway by the analysis of conditional β -catenin loss- and gain-of-function mice.

That a single cell, the fertilized egg, can generate a complete animal with a wide variety of complex tissues and organs is the most remarkable process in nature. The rise of multicellular animal life was a huge evolutionary step that apparently only happened once, as evidenced by the genomic content of today's species. The molecular foot-

prints of evolution can be seen both between species and within them; a small number of signaling pathways are used over and over again, to build similar structures in different organisms as well as to create a wide variety of structures within a single animal's body. Such core signaling pathways include Wnt, Hedgehog, Tgf- β /Bmp, Notch, and receptor tyrosine kinases and are widely conserved in vertebrates and invertebrates (for review, see Beddington and Robertson 1999; Jessell and Sanes 2000; Capdevila and Izpisua Belmonte 2001; Logan and Nusse 2004; Schier and Talbot 2005). They act in parallel or in sequence in order to regulate crucial aspects of development and morphogenesis including cellular proliferation, differentiation, cell migration, and apoptosis. Deregulation of these signaling cascades may lead to severe developmental perturbations and lethality. In adult organisms, the same pathways play crucial roles in the maintenance of tissue homeostasis and control stem cell functions. Their dysfunction may lead to pathological conditions such as cancer (for review, see Hunter 1997; Polakis 2000; Chari and McDonnell 2007; Dreesen and Brivanlou 2007). Almost three decades of interdisciplinary research have revealed the physiological function of these pathways in a spectrum of model organisms including hydra, worms, flies, fish, frogs, mice, and humans. Mice have proven to be a particularly powerful model to study molecular mechanisms of development and disease. This is due to the close evolutionary relationship of rodents and humans, similarities in physiological and pathophysiological mechanisms in mice and man, and the large repertoire of genetic techniques available to assess gene functions in mice.

Wnt signaling is an ancient and highly conserved pathway (for review, see Logan and Nusse 2004; Clevers 2006; Klaus and Birchmeier 2008). The molecular components of Wnt signaling and the mechanisms of Wnt signal transduction have been elucidated through genetic and biochemical experiments in various model organisms and in cultured cells. In 1982, Nusse and Varmus identified the proto-oncogene int-1 (integration-1) as a preferential site for proviral integration of the mouse mammary tumor virus (MMTV) in a mouse model of

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mammary cancer (Nusse and Varmus 1982). Int-1 turned out to be the mammalian homolog of the genetically characterized segment polarity gene in *Drosophila*, wingless (wg) (Nusslein-Volhard and Wieschaus 1980; Cabrera et al. 1987; Rijsewijk et al. 1987). The name currently used for these molecules, "Wnt," is a fusion of the two terms, "int" and "wingless," and refers to the extracellular ligands of the pathway (Nusse et al. 1991). In the late 1980s and early 1990s, many components of the Wnt signaling cascade were discovered through other *Drosophila* mutations, which produced segment polarity defects like wingless, such as the cytoplasmic and nuclear signal transducing molecules Dishevelled, Armadillo/ β -catenin, Pangolin/Lef1, and the characterization of the epistatic relationship between the genes that encode these molecules (McMahon and Moon 1989; Dominguez et al. 1995; Guger and Gumbiner 1995; Behrens et al. 1996). *Xenopus* oocytes provided an additional, widely used assay to study components of the Wnt signaling system. The injection of *Xenopus* oocytes with RNA that encodes components regulating the Wnt pathway in a positive manner—e.g., Wnt1, β -catenin, dominant-negative GSK3 β , or Lef1—resulted in axis duplication (McMahon and Moon 1989; Dominguez et al. 1995; Guger and Gumbiner 1995; Behrens et al. 1996), whereas injection of the RNA of Wnt signaling inhibitors such as Axin1 or Axin2/Conductin resulted in a loss of the primary body axis (Zeng et al. 1997; Behrens et al. 1998). Wnt signaling's contribution to the establishment of the anterior–posterior (A–P) axis was analyzed by gene ablation in the mouse, which demonstrated the conserved function of Axin1 and β -catenin in the Wnt pathway (Zeng et al. 1997; Huelsken et al. 2000).

Our current knowledge of the molecular components of the Wnt signaling cascade is summarized in Figure 1. The signal is initiated by Wnt ligands (there are 19 Wnt genes in the human genome). The secretion and post-translational modifications of Wnt ligands are accomplished by the retromer complex and accompanying molecules, such as porcupine and Wntless (Wls) (for review, see Coudreuse and Korswagen 2007; Hausmann et al. 2007). Wnts bind to and activate specific receptors, members of the Frizzled and LRP families (for review, see Mikels and Nusse 2006; Huang and He 2008). Inhibitors of Wnt–receptor interactions are secreted frizzled-related proteins (sFRP), Dickkopfs (Dkk), and Wnt-inhibitory factor (WIF) (Finch et al. 1997; Leyns et al. 1997; Hsieh et al. 1999). The signal transduction cascades downstream from the Wnt receptors comprise a large number of molecules that signal through different branches and effector molecules (see the Wnt genes homepage at <http://www.stanford.edu/~rnusse/Wntwindow.html>). These include the canonical Wnt/ β -catenin branch (Fig. 1, blue and red in the center), and the noncanonical Jun kinase and the Wnt/ Ca^{2+} branches (Fig. 1, green and orange on the right; Veeman et al. 2003; Kuhl 2004; Logan and Nusse 2004; Seifert and Mlodzik 2007). Central to canonical Wnt signaling is the control of the stability of its core component, β -catenin (Fig. 1, red; Munemitsu et al. 1995). β -Catenin has dual functions

and can form a stable complex with the cell adhesion molecules of the cadherin family, participating in formation of adherens junctions (Fig. 1, top left and right; Takeichi 1991; Kemler 1993; Huelsken et al. 1994; Huber et al. 1997). In the absence of Wnt signals, β -catenin that is not bound in a complex with cadherins is captured by a destruction complex containing GSK3 β , Axin/Conductin, and APC, where it is first phosphorylated, then ubiquitinated on N-terminal sequences, and subsequently targeted to proteasomes that mediate its degradation (Fig. 1, left; Aberle et al. 1994; Salomon et al. 1997; Behrens et al. 1998; Marikawa and Elinson 1998; Liu et al. 2002; Yanagawa et al. 2002). In contrast, in the presence of the Wnt ligands, the destruction complex is disassembled through phosphorylation of LRP5/6 and binding of Axin to LRP, which prevents β -catenin degradation (Mao et al. 2001; Bilic et al. 2007; Schwarz-Romond et al. 2007). Free β -catenin (Fig. 1, red in the center) can then enter the nucleus, where it binds to the Tcf/Lef family of transcription factors, replacing the transcriptional inhibitor Groucho, and activates transcription of Wnt target genes (Behrens et al. 1996; Molenaar et al. 1996; Zeng et al. 1997; Roose et al. 1998; Tetsu and McCormick 1999; for review, see Nelson and Nusse 2004; Clevers 2006; Willert and Jones 2006; see Wnt genes homepage <http://www.stanford.edu/~rnusse/wntwindow.html>).

In line with the initial discovery of Wnt1 in mouse mammary neoplasias, the constitutive activation of Wnt signaling causes tumor formation and progression in humans. Gain-of-function mutations of activators of the Wnt pathway can have oncogenic activity, whereas loss-of-function mutations of inhibitors of Wnt signaling can lead to a loss of tumor suppressor activity. For instance, mutations in the β -catenin gene that enhance its stability, or mutations in genes that control β -catenin stability like APC, the Axins, or E-cadherin, cause various types of human cancers (for review, see Birchmeier and Behrens 1994; Bienz and Clevers 2000; Polakis 2000; Fodde and Brabletz 2007).

Over nearly two decades, gene targeting techniques have provided ever deeper insights into Wnt activity and functions. In mice, these methods are based on homologous recombination in embryonic stem cells and allow the introduction of mutations into any gene (work for which Mario R. Capecchi, Martin J. Evans, and Oliver Smithies were awarded the Nobel Prize in Physiology or Medicine in 2007) (Evans and Kaufman 1981; Doetschman et al. 1987; Thomas and Capecchi 1990). These mutant embryonic stem cells are then used to produce mice that carry the mutant allele. The first targeted mutations in Wnt genes of mice, today commonly referred to as "knockout mouse models," were indeed reported in 1990: In parallel, the laboratories of Mario Capecchi and of Andy McMahon and Alan Bradley had introduced null mutations of the int-1 (Wnt1) gene, which produced developmental changes in the midbrain and cerebellum (McMahon and Bradley 1990; Thomas and Capecchi 1990). During the 1990s, knockout mice carrying targeted mutations in genes encoding newly identified members of the Wnt family of ligands were

Grigoryan et al.

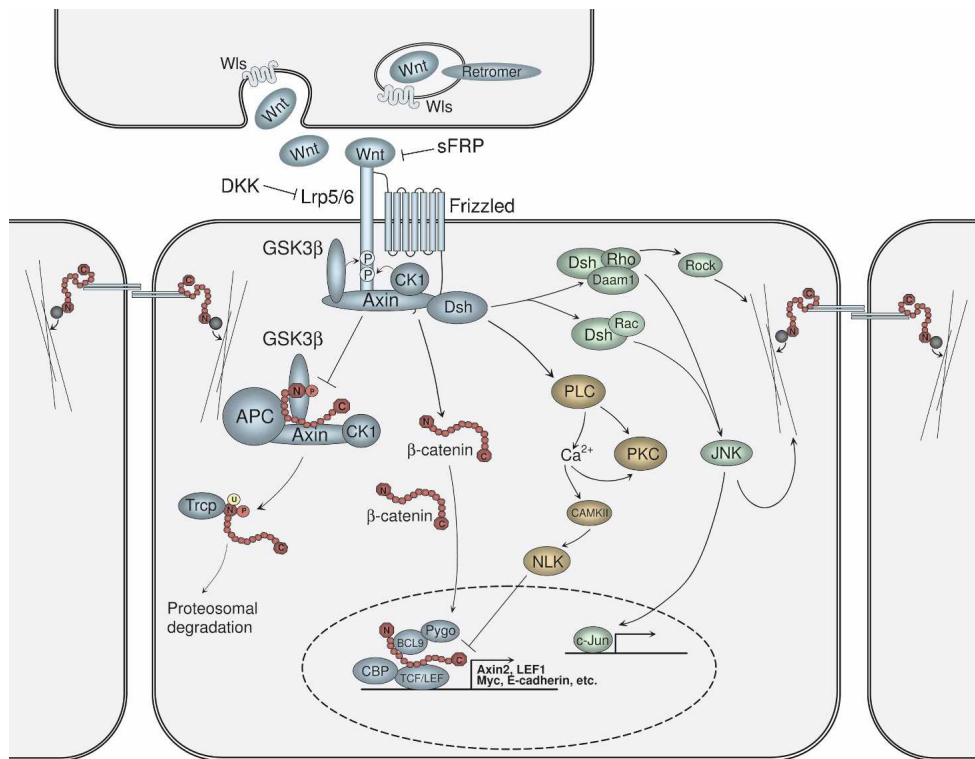


Figure 1. Scheme of the Wnt signaling pathway. The Wnt glycoproteins are produced by the neighboring cells and secreted in a process that involves many components, such as a multiprotein retromer complex, and the multispanning transmembrane protein Wntless (Wls). The secreted Wnt ligands bind to the receptors of the seven-membrane-spanning Frizzled family and single-spanning LRP family. Secreted factors like secreted Frizzled-related proteins (sFRPs) bind to Wnts and block the interaction downstream with Frizzled receptors. Dickkopf antagonizes Wnt signaling by blocking LRP receptors. Three main branches are activated downstream from the Wnt signals: canonical Wnt/ β -catenin signaling (blue and red, in the center and on the left), and the noncanonical Jun kinase and Wnt/ Ca^{2+} pathways (green and orange on the right). The central component of the canonical Wnt signaling is β -catenin (red). (Left and right) In the absence of the Wnt signals, β -catenin forms a stable complex with E-cadherin and participates in adherens junction formation. (Left) Excess β -catenin is captured by a destruction complex containing GSK3 β , Axin1, Axin2/Conductin, and APC, where it gets N-terminally phosphorylated and, following ubiquitination by β -Trcp, targeted to proteasome-mediated degradation. In contrast, in the presence of the Wnt ligands, disassembly of the destruction complex occurs through phosphorylation of LRP5/6 by GSK3 β and CK1 α , and binding of Axin to LRP, thereby preventing β -catenin degradation. Free cytoplasmic β -catenin can then enter the nucleus, where it forms a transcriptionally active complex with Tcf/Lef family of transcription factors, by replacing Grouchos from chromatin, and interacting with other coactivators like CBP, BCL9, and Pygopus. The three pathways branch at the level of Dishevelled (Dsh) protein. In the noncanonical Jun kinase pathway, Dsh via Daam1, is connected to downstream effectors, such as Rho, and regulates cytoskeletal organization and cell polarity. The Wnt/ Ca^{2+} pathway leads to the release of intracellular calcium, and involves activation of Phospholipase C (PLC) and protein kinase C (PKC). Ca^{2+} can activate Calmodulin-dependent protein kinase C (CaMKII), and Nemo-like kinase (NLK), and suppress canonical Wnt signaling.

generated (Stark et al. 1994; Takada et al. 1994; Fritzsche et al. 1995; Parr and McMahon 1995; Yamaguchi 2001; for review, see van Amerongen and Berns 2006). These studies revealed an important role of Wnt genes in development of additional tissues including the mesoderm, kidney, and extremities. The fact that compound mutations of these genes often exhibited stronger and earlier phenotypes than the single mutations suggested a functional redundancy between the different Wnt genes (Ikeya et al. 1997; Ikeya and Takada 1998; Yamaguchi et al. 2005). Identification of β -catenin as the one nonredundant and central component of the canonical Wnt pathway, a bottleneck through which all signals pass, made it a particularly attractive target for genetic manipulation. Null mutations of β -catenin result in gastrulation defects and embryonic lethality (Haegel et al.

1995; Huelsken et al. 2000; Morkel et al. 2003). Such mutant embryos failed to form an A–P axis at embryonic day 6 (E6) and did not generate mesoderm at E7. Apparently, β -catenin does not play an essential earlier role in Wnt signaling; e.g., in embryonic stem cells or blastocyst formation (but see also Doble et al. 2007; Cole et al. 2008). Importantly, β -catenin-deficient embryos preserved intact adherens junctions, since plakoglobin (γ -catenin), another member of armadillo protein family, can take over the role of β -catenin in cell adhesion (Huelsken et al. 2000). However, early constitutive activation of β -catenin in mouse embryos leads to a change of the cell fate in the epiblast (Morkel et al. 2003; Kemler et al. 2004). We should also mention that the use of the various Wnt reporter mice generated by several groups was an important help to the field, and those mice were

used extensively to monitor endogenous Wnt signaling activity (DasGupta and Fuchs 1999; Cheon et al. 2002; Lustig et al. 2002; Soshnikova et al. 2003; Bridgewater et al. 2008).

The ability to mutate genes in the mouse in a temporal and tissue-specific manner by conditional gene mutation, techniques developed by Klaus Rajewsky and collaborators (Gu et al. 1994), has permitted both overcoming the early embryonic lethality of the β -catenin mutation and accurate studies of the role of canonical Wnt signaling in various tissues at many developmental stages. This strategy relies on the introduction of recombinase recognition sites (LoxP or Frt) that flank the target gene or parts of it. These recognition sites are, for instance, inserted into intronic sequences of a gene in such a way that in the absence of the recombinase (Cre or Flp), the normal gene function is not altered. However, tissue-specific expression of Cre or Flp leads to a recombination event, resulting in the excision of the flanked ("floxed" or "Flrted") part of the gene and, as a consequence, the alteration or elimination of gene function (for review, see Lewandoski 2001; Yu and Bradley 2001). In the last de-

cade, two different approaches have been applied in order to conditionally mutate β -catenin in mice: The first, a deletion of several exons of the β -catenin gene, resulted in tissue-specific null mutations and loss of canonical Wnt signaling (Fig. 2a). The second, a deletion of a single exon of the β -catenin gene, exon 3, resulted in the production of an N-terminally truncated and stabilized form of the protein, which constitutively activates Wnt signaling (Fig. 2b). In order to generate conditional null mutations of β -catenin, two alleles were generated in which about a third of the β -catenin gene is flanked by loxP sites (Fig. 2a): in the β -catenin^{Wbm/flox} allele produced by our laboratory (Jackson laboratory nomenclature: $Ctnnb1^{tm4/Wbm}$), the loxP sites flank exons 3–6 (Huelken et al. 2001); in the β -catenin^{Kem/flox} allele produced by Kemler's laboratory (Brault et al. 2001) ($Ctnnb1^{tm2/Kem}$), loxP sites flank exons 2–6. Cre-mediated recombination produced loss-of-function mutations of β -catenin in a tissue-specific manner (Fig. 2c). In order to generate a conditional gain-of-function mutation of β -catenin, the Taketo laboratory introduced loxP sites that flank exon 3, β -catenin^{Mmt/flox} ($Ctnnb1^{tm1/Mmt}$) (Fig. 2b; Harada et

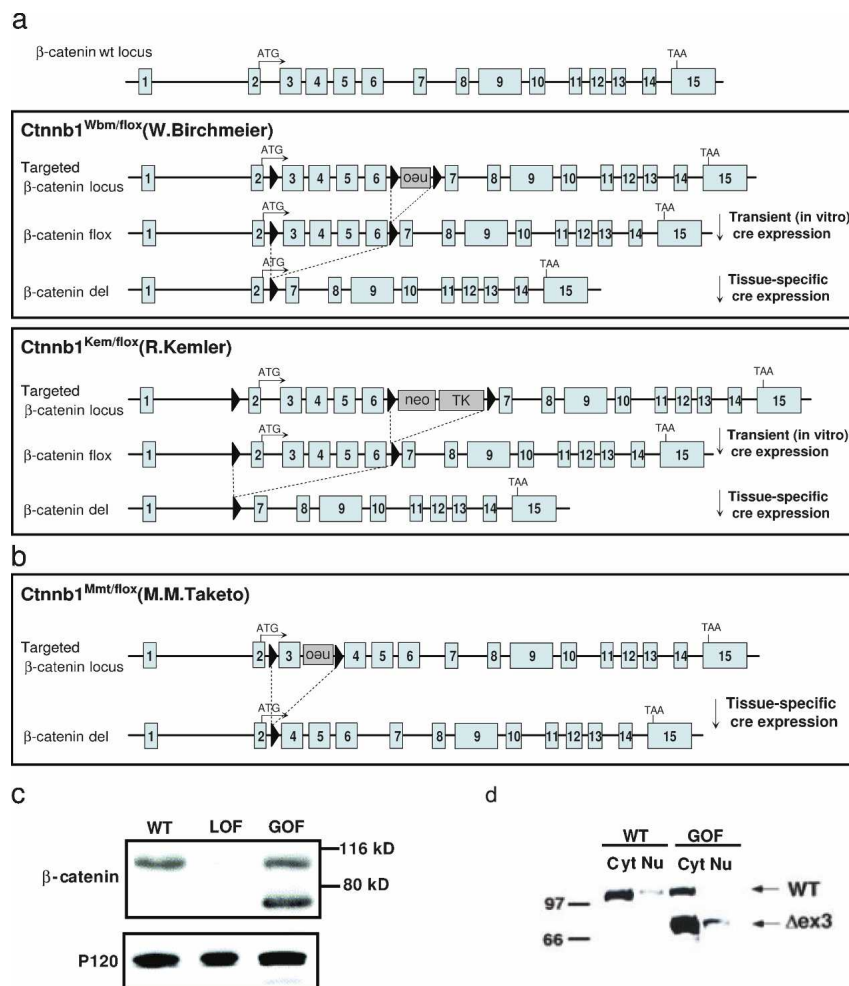


Figure 2. Introduction of conditional loss- and gain-of-function mutations into the β -catenin locus. (a) Genomic locus of the β -catenin gene and the two strategies of generation of the β -catenin loss-of-function alleles. In one strategy, loxP sites flank exons 3–6, to generate the $Ctnnb1^{Wbm/flox}$ allele; in the second strategy, loxP sites flank exons 2–6 to generate the $Ctnnb1^{Kem/flox}$ allele. In both cases, the floxed parts encode the N-terminal domain and the Armadillo repeats 1–4 of the β -catenin protein. Transient expression of cre recombinase results in excision of the neo resistance cassette. Subsequently, tissue-specific cre expression leads to excision of the floxed exons, frameshift mutations, and production of a null-allele (see in c, cf. wild type with LOF). (b) Generation of the β -catenin gain-of-function allele. LoXP sites flank exon 3, which encodes phosphorylation sites targeted by GSK3 β . Tissue-specific cre expression leads to excision of exon 3 and in-frame splicing on exons 2 and 4, thus resulting in expression of a protein that escapes the degradation. (c,d) Western blot analysis showing tissue-specific recombination of the targeted loci. In the conditional loss-of-function mutant, no β -catenin protein can be detected, whereas in the gain-of-function mutants, a shortened β -catenin variant is produced (cf. wild type, LOF, and GOF). On the right, the expression of the mutant β -catenin protein after cre-mediated recombination in cytoplasmic (Cyt) and nuclear (Nu) fractions of the recombinant embryonic stem cells is shown. (WT) Wild type; (LOF) loss-of-function allele; (GOF) gain-of-function allele.

and nuclear (Nu) fractions of the recombinant embryonic stem cells is shown. (WT) Wild type; (LOF) loss-of-function allele; (GOF) gain-of-function allele. d was reprinted with permission from Macmillan Publishers Ltd: Harada et al. (1999), © 1999; and c was reprinted from Zechner et al. (2003) with permission from Elsevier.

Grigoryan et al.

al. 1999). Exon 3 of the β -catenin gene encodes the N-terminal part of the protein containing serine (S) and threonine (T) residues (S45, T41, S37, S33) that are phosphorylated by CK1 α and GSK3 β (Amit et al. 2002; Liu et al. 2002; Yanagawa et al. 2002). Cre-mediated recombination excises exon 3 and leads to an in-frame fusion of exon 2 and 4 sequences, which encodes a shortened version of β -catenin lacking N-terminal phosphorylation and ubiquitination sites (Fig. 2c,d; Harada et al. 1999; Zechner et al. 2003). These alleles, which allow conditional inhibition (loss-of-function) or activation (gain-of-function) of Wnt/ β -catenin signaling, provided powerful tools to analyze Wnt functions in mammalian development (Table 1).

Wnt/ β -catenin signaling in skin and teeth

By the mid- and end-1990s, several lines of evidence indicated a role of Wnt/ β -catenin in development of the skin and skin appendages. The epidermis forms the outermost layer of the mammalian skin. Progenitor cells in the basal layer of the epidermis constantly proliferate, ensuring the renewal of the epithelium. In addition to these epidermal progenitors, the bulge attached to the hair follicles is a niche for skin stem cells. These are able to contribute to all epithelial cell types of the skin appendages; e.g., hair follicles and glands (Fig. 3a, stem cells are marked with K15 and β 1 integrin probes, arrows; Huelsken et al. 2001). After wounding, bulge stem cells contribute in addition to the newly formed epidermal epithelium (for review, see Paus and Foitzik 2004; Blanpain and Fuchs 2006). Several Wnt genes are expressed in the skin (Wang and Shackleford 1996; Saitoh et al. 1998; for review, see Millar et al. 1999). Early experiments, in which Wnt3, Lef1, or activated β -catenin were overexpressed in the skin epithelium, had indicated a role of the Wnt/ β -catenin pathway in development of the hair follicles (Zhou et al. 1995; Gat et al. 1998; Millar et al. 1999; Kishimoto et al. 2000). In addition, gene ablation had demonstrated an essential role of Lef1 in formation of hair, whiskers, and other epidermal appendages (van Genderen et al. 1994). Conversely, one-third of human sebaceous gland tumors carry inactivating mutations of Lef1, indicating that Wnt signaling activity needs to be repressed for proper sebaceous gland development (Takeda et al. 2006).

By conditional mutation of β -catenin in the skin, using cre driven by the cytokeratin (K) 14 promoter, we showed that canonical Wnt signals are crucial for the generation of hair follicles (Table 1; Huelsken et al. 2001). The epidermis develops correctly, but the skin of the mutant mice becomes naked. Instead of hair, unusual cyst-like structures appeared in the skin of these mice (Fig. 3b). The multilayered epithelium surrounding the cysts still contained stem cells (stained with K15 and β 1 integrin, arrows in Fig. 3b), and was positive for epidermal markers but negative for hair follicle markers (Huelsken et al. 2001). Postnatal deletion of β -catenin leads to loss of quiescence and stem cell character of the bulge stem cells (Lowry et al. 2005). Altogether, these

data show that Wnt/ β -catenin signals control the cell fate of the bulge stem cells: Such signals are required for follicular but not epidermal differentiation (see scheme in Fig. 3e). Moreover, transgenic expression of dominant-negative Lef1 also resulted in the formation of cysts instead of hair (Merrill et al. 2001; Niemann et al. 2002). A similar phenotype—i.e., a lack of hair and other epidermal appendages, such as teeth and mammary glands—was observed after ectopic expression of the Wnt inhibitor Dkk1 in the skin (Andl et al. 2002). Finally, Wnt signals have also been found to be essential for de novo hair follicle formation after wounding (Ito et al. 2007). A converse phenotype, accelerated and excessive formation of hair placodes, was observed when a conditional gain-of-function mutation of β -catenin was introduced by K14 cre in the skin (Närhi et al. 2008). Such animals display densely packed hair follicles with little or no interfollicular epidermis (Fig. 3c,d). This is in line with a previous report of transgenic overexpression of Δ N- β -catenin in the skin; i.e., constitutively active β -catenin, which resulted in a de novo formation of hair follicles and in the generation of hair follicle tumors (Gat et al. 1998; Chan et al. 1999). That β -catenin plays a minor role in epidermal development, in contrast to hair, was corroborated using cultured epidermal keratinocytes isolated from the skin of β -catenin^{Kemflox} mice, in which the loss-of-function mutation was introduced by Adenovirus-mediated gene transfer of cre (Posthaus et al. 2002). In such cells, proliferation, cell cycle progression, and expression of CyclinD1 were similar to the processes in control cells. Furthermore, β -catenin-null mutant keratinocytes remained adhesion-competent, due to the functional substitution of β -catenin by plakoglobin (γ -catenin). More recently, interactions between Wnt and Bmp signaling have been described as essential in the control of the skin stem cell niche (Zhang et al. 2006; Kobiela et al. 2007). The conditional ablation of Bmp receptor 1a (BmpR1a) resulted in increased canonical Wnt signaling, stem cell overproliferation and expansion of the bulge, and loss of quiescent cells. Oscillating expression of Bmps in normal skin regulate oscillations in Wnt signaling in cases such as when Bmp levels in the dermis are high, Wnt signaling activity is low and vice versa (Plikus et al. 2008). The oscillations of Bmp and Wnt signaling ensure periodic activation of the bulge stem cells and allow the coordinated hair differentiation during the hair cycle. Finally, the function of Wnt/ β -catenin signaling has also been studied in the skin mesenchyme by conditional loss- and gain-of-function mutations of β -catenin, using an engrailed-1 cre or HoxB6 cre allele (Atit et al. 2006; Ohtola et al. 2008). This demonstrated an essential role of canonical Wnt signals in the specification of the dermis (Table 1).

Loss- and gain-of-function mutations of β -catenin, using K14 cre, have also allowed the analysis of canonical Wnt signals in the development of teeth, another epidermal appendage (Table 1; Järvinen et al. 2006; Liu et al. 2008). Canonical Wnt signals are required in the epithelium at several steps during the formation of teeth, first at an early stage for proper tooth bud development, and

Table 1. Loss- and gain-of-function mutations of β -catenin in mice

Organ ^a	Cre line	Cre expression	β -catenin	Phenotype	References
Bone/ cartilage	Prx1 cre	Osteo- chondroprogenitors	LOF	Reduced osteoblastogenesis, increased chondrogenesis	Akiyama et al. 2004; Guo et al. 2004; Day et al. 2005; Hill et al. 2005; Glass et al. 2005; Rodda and McMahon 2006
	Dermo1 cre		GOF	Increased osteoblastogenesis, reduced chondrogenesis	
	Col2a1 cre	Osteoblast progenitors	LOF	Low bone mass and high resorption	
	Colla cre		GOF	Increased bone mass and low resorption	
	Osx1-GFP cre ^b	Osteoblast progenitors	LOF	Defective osteoblast maturation	
GOF	Accelerated osteoblast maturation				
CNS	Brn4 cre	CNS	LOF	Reduction of the progenitor pool, loss of dI2 and dI3 interneurons	Ille et al. 2007; Zechner et al. 2003, 2007
			GOF	Expansion of the progenitor pool, expansion of dI2 and dI3 interneurons	
	POMC cre ^c	Cerebral cortex and hippocampus	LOF	Abnormal neuronal maturation and dendritic morphology	Gao et al. 2007
	Nestin cre	Cerebellum	LOF	Mid-brain and cerebellum abnormalities	Schuller and Rowitch 2007
	Emx 1 cre	Telencephalon Cerebral cortex and hippocampus	LOF	Increased seizure susceptibility	Campos et al. 2004 Machon et al. 2003
	D6 cre		LOF	Impaired proliferation of hippocampus progenitors	
	HSA cre ^d	Myotome	LOF	Impaired differentiation of neuron-muscular junctions	Li et al. 2008
	Nes8 cre	Telencephalon	LOF	Down-regulation of dorsal markers, up-regulation of ventral markers	Backman et al. 2005
GOF			Up-regulation of dorsal markers, down-regulation of ventral markers		
Ear	Pax2 cre	Otic precursors	LOF	Reduction of the otic placode	Ohyama et al. 2006
			GOF	Expansion of the otic placode at the expense of epidermis	
Eye	Lens cre	Lens surface ectoderm	LOF	Abnormal lens morphology, ectopic lens	Smith et al. 2005; Miller et al. 2006; Kreslova et al. 2007
	LR cre ^e	Lens ectoderm, partially in retina	GOF	Loss of lens structure	
	Six3 cre	Developing retina	LOF	Abnormal eye morphology, ectopic lens in the pericocular area	Fu et al. 2006
	α cre	Anterior-peripheral retina	LOF	Disruption of the retinal laminar structure, loss of retinal progenitor orientation and arrangement	
Heart	Mesp1 cre ^f	All heart progenitors	GOF	Loss of proper retinal structure, transdifferentiation of neural cells into pigmented cells	Kioussi et al. 2002; Lickert et al. 2002; Liebner et al. 2004; Chen et al. 2006; Ai et al. 2007; Baurand et al. 2007; Cohen et al. 2007; Klaus et al. 2007; Kwon et al. 2007; Lin et al. 2007; Qu et al. 2007; Qyang et al. 2007; Zamora et al. 2007; Zhou et al. 2007
	Nkx2.5 cre Sm22 α cre ^g	All heart precursors Cardiomyocytes and endothelial cells	LOF	Defects in second heart field proliferation	
			GOF	Disrupted heart tube formation	
	Isl1 cre	Second heart field	LOF	Defects in outflow tract and right ventricle	
			GOF	Enlarged size of second heart field derivatives due to enhanced proliferation of the second heart field	
	Mef2c-AHF cre ^h	Second heart field	LOF	Defects in outflow tract and right ventricle	
α MHC cre	Cardiomyocytes	Het-LOF	Shortened right ventricle, dense outflow tract myocardium Attenuated cardiac hypertrophy following pathological stress		

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Grigoryan et al.

Table 1. (continued)

Organ ^a	Cre line	Cre expression	β -catenin	Phenotype	References
	Wnt1 cre	Cardiac neural crest cells	LOF	Defects in outflow tract remodeling	
	Tie2 cre	Endothelial cells	LOF	Defects in endocardial cushion formation	
	GATA5 cre	Epicardium	LOF	Impaired coronary artery formation	
	K19 cre	Embryonic endoderm	LOF	Multiple heart formation	
	α MHC cre ¹	Cardiomyocytes	LOF	Cardiac hypertrophy	
Hemato- poietic system	Lck cre	Thymocytes	GOF	Impaired T-cell development	Gounari et al. 2001;
	Mx cre	Hematopoietic precursors	LOF	Impaired HSC self-renewal, no effect in combination with plakoglobin	Mulroy et al. 2003; Xu et al. 2003; Scheller et al. 2006; Zhao et al. 2007;
	Vav cre	Hematopoietic precursors	GOF LOF	Loss of quiescent HSCs Prevention of CML development	Jeannet et al. 2008; Koch et al. 2008
Intestine	Cyp450 cre	Intestinal epithelium	LOF	Loss of crypts	Harada et al. 1999; Ireland et al. 2004; Fevr et al. 2007
	Villin cre ^{ERT1}		GOF	Intestinal polyposis	
	K19 cre	Intestinal crypts	GOF	Intestinal adenoma formation	Sangiorgi and Capecchi 2008
	Fabpl cre				
Bmi1 cre					
Kidney	Six2GFP cre	Kidney mesenchyme	LOF GOF	Reduced kidney size and branching Increased kidney size, absent branching	Park et al. 2007; Bridgewater et al. 2008; Marose et al. 2008
	Hoxb7 cre	Kidney epithelium	LOF GOF	Hypoplasia, loss of UB Increased differentiation	
Limb	Brn4 cre	AER	LOF	Limb malformations, defects in D-V and P-D patterning	Soshnikova et al. 2003
			GOF	Enlarged limbs, D-V patterning defects	
	Msx2 cre	AER	LOF	Loss of hindlimbs, truncated forelimbs, disrupted D-V patterning	Barrow et al. 2003
Prx1 cre	Limb mesenchyme	LOF	Shortened limbs	Hill et al. 2006	
		GOF	Premature AER regression		
Liver	Albumin cre	Hepatocytes	LOF	Smaller liver size, normal recovery of liver mass after hepatectomy, impaired ammonia detoxification	Sekine et al. 2006, 2007; Tan et al. 2006
	Fabpl cre	Hepatocytes	GOF	No liver carcinogenesis	Harada et al. 2002
	Viral cre	Mosaic in liver and gut			
	Foxa3 cre	Foregut and liver endoderm	LOF	Defects in hepatoblast expansion and maturation	Tan et al. 2008
α FP-Albumin cre ^k	Postnatal hepatocytes	LOF	Impaired liver growth	Apte et al. 2006, 2007	
Lung	SP-C cre ¹	Lung epithelium	LOF	Inhibition of distal airways, enlargement of proximal airways	Mucenski et al. 2003, 2005; Shu et al. 2005; De Langhe et al. 2008; Yin et al. 2008; Zhang et al. 2008
	CCSP cre ^m		GOF	Enlargement of distal airways, inhibition of proximal airways, increased amount of bronchioalveolar stem cells	
	Dermo1 cre	Lung mesenchyme	LOF	Loss of lung muscle progenitors, impaired lung growth	
Mammary gland	WAP cre MMTV cre	Mammary epithelium	GOF	Mammary hyperplasia, precocious differentiation	Miyoshi et al. 2002; Renou et al. 2003
Neural crest	Wnt1 cre	Neural crest progenitors	LOF	Loss of mid-brain and part of hindbrain, loss of cranial bone and cartilage, lack of sensory neurons and melanocytes, loss of Schwann cells	Brault et al. 2001; Hari et al. 2002; Lee et al. 2004
			GOF	Expansion of sensory lineage	

continued on next page

Table 1. (continued)

Organ ^a	Cre line	Cre expression	β -catenin	Phenotype	References
Pancreas	Pdx1 cre	Pancreas progenitors	LOF	Reduction of exocrine and endocrine compartments	Dessimoz et al. 2005; Murtaugh et al. 2005;
	Elastase cre	Exocrine pancreas	GOF	Increased exocrine compartment	Heiser et al. 2006;
	RIP cre	Pancreatic β cells	LOF	Reduced exocrine compartment	Rulifson et al. 2007;
Pituitary	Pitx1 cre	Radthke's pouch progenitors	GOF	Increased β -cell proliferation	Wells et al. 2007
	Pit1 cre	Sublineages of the pituitary	LOF	Loss of Pit1-dependent cell types Absence of the pituitary No effect on cell differentiation	Olson et al. 2006
Reproductive system	MisrII cre ⁿ	Müllerian duct mesenchyme	LOF	Switch from myogenesis to adipogenesis	Arango et al. 2005; Deutscher et al. 2007
	Amhr2 cre ^o		LOF	Uterine horn hypotrophy and loss of oviduct coiling	
	AMH cre ^p	Sertoli cells	LOF	No effect on testis/Sertoli cell development	Chang et al. 2008
	MMTV cre	Prostate epithelium	GOF	Testicular cord disruption and germ cell loss Hyperproliferation and metaplasia of prostate epithelium	Bierie et al. 2003
Skin	K14 cre	Epidermis and teeth	LOF	Loss of hair follicles	Gat et al. 1999; Huelsken et al. 2001; Ito et al. 2007; Malanchi et al. 2008
	K14 ^{ERT} cre ^d		GOF	De novo hair generation, tumor formation after wounding	
	Engrailed 1 cre	Central dermomyotome	LOF	Tumor regression, loss of cancer stem cells, prevention of de novo hair follicle generation after wounding	
	HoxB6 cre	Lateral plate mesoderm	LOF	Loss of dorsal and ventral dermis and hair placodes Expansion of dorsal dermis, ectopic dermis	Atit et al. 2006; Ohtola et al. 2008
Somites	Brachyury cre	Mesoderm progenitors	LOF	Lack of the ventral dermis and ventral body wall	
			GOF	Loss of posterior paraxial mesoderm, abnormal segmentation Expanded posterior paraxial mesoderm, abnormal segmentation	Aulehla et al 2008; Dunty et al 2008
Stomach/esophagus	Shh cre	Stomach endoderm	GOF	Reduced stomach size, inverted positioning of cell types of the esophagus, posteriorization of the gut tube	Kim et al. 2005
Taste buds	K14 cre	Sensory epithelium	LOF	Loss of fungiform taste placodes	Liu et al. 2007
Tooth	K14 cre	Tooth progenitors	GOF	Ectopic fungiform placodes	
			GOF	Supernumerary tooth generation	Järvinen et al. 2006

^aTissues and organs are listed alphabetically.^bOsterix 1 cre.^cProopiomelanocortin cre.^dHuman skeletal actin cre.^eCre is expressed in lens, and partially in retina.^fMesoderm posterior cre.^gSmooth muscle 22 α cre.^hMyocyte enhancer factor 2C anterior heart field enhancer and promoter (AHF) cre.ⁱInducible α -myosin light chain cre.^jTamoxifen-inducible Villin cre.^k α -fetoprotein enhancer-albumin cre.^lInducible surfactant protein C cre.^mClara cell-specific protein cre.ⁿMüllerian inhibiting substance type II receptor cre.^oAnti-Müllerian hormone receptor 2 cre.^pAnti-Müllerian hormone cre.^qTamoxifen-inducible K14 cre.

Grigoryan et al.

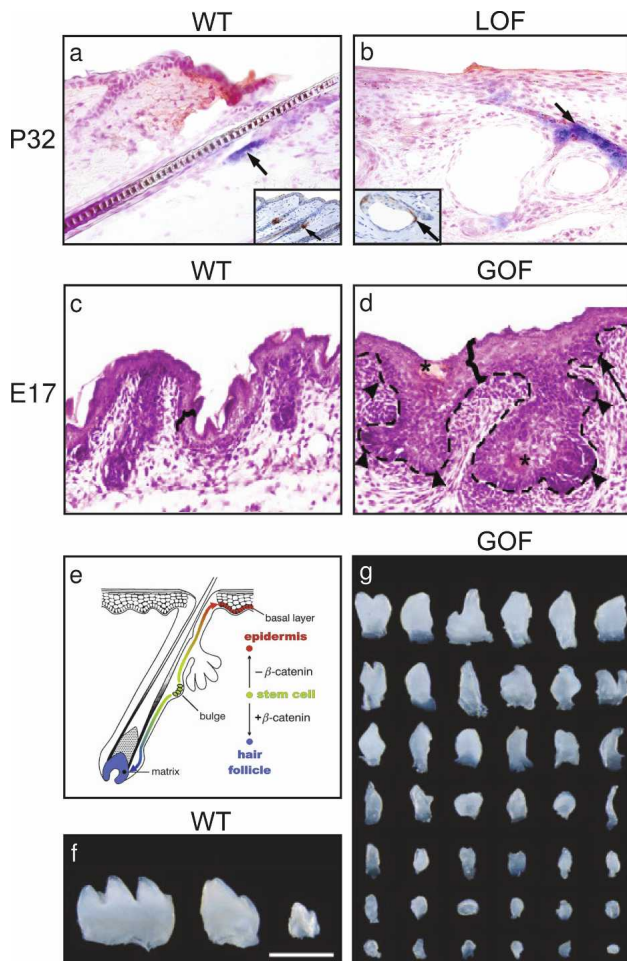


Figure 3. Wnt/ β -catenin signaling in skin and teeth. (a,b) Conditional loss-of-function mutations of β -catenin in skin prevent hair follicle generation and result in formation of cysts at P32. Stem cells are localized to the specific areas close to the cysts in β -catenin loss-of-function mutants, as shown by in situ hybridization for the stem cell marker Keratin 15 (arrows) and immunohistochemistry for β 1-integrin (insets, arrows). (c,d) Conditional gain-of-function mutations of β -catenin in skin result in disturbed hair follicle morphogenesis at E17, which is accompanied by epidermal thickening (brackets) and the formation of additional hair placodes (d, arrows and arrowheads), as well as in keratin deposits (d, asterisks). (e) Scheme of the role of β -catenin in fate decisions of the bulge stem cells toward follicular or epidermal lineages. (f,g) Conditional gain-of-function mutations of β -catenin in tooth epithelium lead to generation of supernumerary teeth of various developmental stages and sizes from one mutant molar tooth bud. a, b, and e are reprinted with permission from Huelsken et al. (2001) with permission from Elsevier. c and d are reprinted by permission of the Company of Biologists from Närhi et al. (2008). f and g are reprinted with permission from Järvinen et al. (2006); © 2006 National Academy of Sciences, U.S.A.

at later stages for patterning of the molar cusp and for establishment of molar polarity (Liu et al. 2008). Wnt signaling is also essential in tooth renewal (Järvinen et al. 2006): Conditional gain-of-function mutations of β -catenin in tooth epithelium resulted in continuous for-

mation of teeth, and in generation of supernumerary teeth (>40 teeth from one tooth germ) (Fig. 3f,g). It remains to be determined whether a stem cell population exists in the tooth germs of the dental epithelium and if so, what role Wnt signaling plays in such cells. Canonical Wnt signaling is not only required in the epithelium, but also in the mesenchyme of skin and its appendages, as demonstrated by the analysis of *Lef1* mutant and *Dkk1*-transgenic mice (van Genderen et al. 1994; Andl et al. 2002; Sasaki et al. 2005; Liu et al. 2008). This fits given the fact that epithelial–mesenchymal interactions are known to govern hair and tooth development.

Just as proper canonical Wnt signaling guides healthy development in these tissues, defects play a role in tumors, as revealed by the analysis of loss- and gain-of-function mutations in β -catenin. Cancer stem cells and normal stem cells share many characteristics, like the capacity for self-renewal and differentiation and their dependence on a particular micro-environment, the (cancer) stem cell niche (for review, see Passegue and Weissman 2005; Wilson and Trumpp 2006; Fodde and Brabletz 2007; Sneddon and Werb 2007). Malanchi et al. (2008) have now identified a population of cancer stem cells in mouse epidermal tumors—i.e., early squamous cell carcinomas—that are dependent on β -catenin and that are phenotypically and functionally similar to normal bulge skin stem cells. These cancer stem cells can be isolated by cell sorting based on the presence of the stem cell marker CD34 and absence of other markers. In normal mouse skin, CD34⁺ bulge stem cells account for ~1.8% of keratinocytes. However, cutaneous tumors, induced by chemical (DMBA/TPA) carcinogenesis or by expression of the Ras oncogene, contain a ninefold increase of the CD34⁺ cell population. The tumorigenic capacity of the CD34⁺ cells was >100-fold greater than that of unsorted cells. Secondary tumors that formed after transplantation resembled the parental tumors. For example, they also contained a small population of CD34⁺ stem cells. Remarkably, the deletion of β -catenin in DMBA/TPA or Ras-induced tumors using an inducible system that allows conditional mutagenesis after the addition of tamoxifen (*K14-cre^{ERT}*, β -catenin^{Wbm/flox}) resulted in complete tumor regression (Malanchi et al. 2008).

Thus, canonical Wnt signals are required in normal skin to instruct bulge stem cells toward the hair cell fate, whereas in epidermal tumors, they control the maintenance of skin cancer stem cells. It may be possible to exploit this differential response of normal and cancer stem cells in therapies. Overall, the analysis of conditional loss- and gain-of-function mutations of β -catenin was fundamental for these discoveries.

Wnt/ β -catenin signaling in the gastrointestinal tract and pancreas

Wnt signaling plays a crucial role during development of the gastrointestinal tract, in particular, in endoderm specification and gut tube patterning, as well as in intestinal stem cell maintenance (Lickert et al. 2002; Gregorieff et al. 2004). During development, the gastrointesti-

nal tract forms from the embryonic gut tube. This structure, which is of endodermal origin, undergoes a series of morphogenic changes and patterning events along its A–P and dorsal–ventral (D–V) axes that give rise to major organs such as the esophagus, stomach, and intestine (small intestine, colon), as well as to associated organs including the liver, pancreas, and thyroid (Wells and Melton 1999; Gregorieff and Clevers 2005). The absorptive epithelium of the small intestine is ordered into villi and crypts of Lieberkuehn; in contrast, colon epithelium consists of crypts only. Intestinal crypts are stem cell niches that ensure the self-renewing capacity of the intestine through maintenance of intestinal stem cells and proliferation of transit amplifying cells, which give rise to all differentiated cell types, that is, enterocytes, enteroendocrine and goblet cells of the villi, and to the Paneth cells, which reside at the bottom of the crypts. Activated Wnt signaling is required in the posterior part of the gut tube for intestinal lineage specification, whereas in the anterior part, stomach and esophagus formation require Wnt repression (Gregorieff et al. 2004; Okubo and Hogan 2004). In the adult intestine, canonical Wnt activity is restricted to the proliferative compartment of crypts only. Remarkably, the crypt progenitor compartment of the small intestine was completely lost in *Tcf4*^{-/-} mice, whereas the differentiated villus epithelium was initially unaffected (Korinek et al. 1998). This demonstrated that downstream from canonical Wnt signaling, the *Tcf4*-driven genetic program is essential to maintain the crypt stem cells of the small intestine.

As in the skin, defects in Wnt signaling in the intestine can lead to cancer. The first gene of the Wnt pathway to be implicated in human cancer was APC (Adenomatous Polyposis coli), a gene that is silenced in 85% of human colon cancers (for review, see Kinzler and Vogelstein 1996; Bienz and Clevers 2000; Polakis 2000; Clevers 2006). Consequently, numerous mouse models have been generated that resembled human colon cancer, such as APC^{Min} and other APC mutants, as well as a mouse that overexpresses N-terminally truncated β -catenin (Moser et al. 1990; Oshima et al. 1995; Wong et al. 1996; Smits et al. 1999). The first conditional gain-of-function mutation of β -catenin related to the formation of the intestinal epithelium was introduced by Taketo and colleagues (Harada et al. 1999), using β -catenin^{Mmt/flox} and K19 cre (Table 1). Numerous polyps in the small intestine (up to 3000 per mouse) were found in all mice at a young age. In contrast to the well-separated polyps in the APC^{Min} model, polyps of the β -catenin mutants were mainly formed in contiguous sheet-like structures, and nascent microadenomas were found in the colon mucosa. Conditional gain-of-function mutations of β -catenin were also introduced postnatally in the intestinal villae, using a cre driven by the fatty acid binding protein gene promoter (*Fabp* cre). The mutant mice developed well-separated polyps at the crypt intervillus region (200–700 per mouse) (Harada et al. 1999). In both models, polyps were composed uniquely of enterocytes. Since the gain-of-function β -catenin mutations closely resembled APC^{Min}, it is likely that APC acts through

β -catenin in adenoma formation (for review, see Clarke 2006). An elegant recent study genetically demonstrated that *c-myc* is an essential downstream target of APC/ β -catenin in the intestine. The conditional ablation of *c-myc* using an inducible Cyp450 promoter-driven cre normalized the tissue; i.e., restored proper proliferation, induced differentiation, and increased crypt size, which were perturbed following conditional loss of *Apc* (Sansom et al. 2007). Conditional loss-of-function mutations of β -catenin were also introduced in the intestinal epithelium using two cre lines, inducible Cyp450 and villin cre (Table 1; Ireland et al. 2004; Fevr et al. 2007). Inactivation of β -catenin resulted in rapid loss of crypt structures, and the increased differentiation of stem cells and transient amplifying cells into enterocytes, similar to the *Tcf4*^{-/-} phenotype (Korinek et al. 1998). Conditional gain-of-function mutations of β -catenin were also introduced in the stomach and esophagus epithelium, using Sonic Hedgehog cre (Table 1; Kim et al. 2005). Mutant embryos showed reduced stomach size and highly disorganized glandular morphology. The esophagus displayed inverted positioning of squamous and cuboidal epithelial cell types along the D–V axis. The esophagus epithelium showed abnormal mucin secretion, which is normally produced by the stomach, indicating posteriorization of the gut tube (Kim et al. 2005, 2007). These data are consistent with the general role of Wnt signaling in the establishment of A–P and D–V polarity of the gut tube.

In summarizing the data on conditional mutagenesis of β -catenin in the intestine, we should mention that many important functions of canonical Wnt signals were identified earlier, in the 1990s; i.e., before conditional mutagenesis of β -catenin was first used in 1999. For instance, much information on the role of Wnt signaling in the intestine was generated by the analysis of APC functions in tumor formation, or the biochemical characterization of the β -catenin/APC and β -catenin/Tcf interaction and their role in Wnt signaling (Bodmer et al. 1987; Leppert et al. 1987; Moser et al. 1990; Su et al. 1992; Munemitsu et al. 1995; Behrens et al. 1996; for review, see Kinzler and Vogelstein 1996; Bienz and Clevers 2000; Polakis 2000; Clevers 2006). The generation of the first β -catenin gain-of-function mutation (β -catenin^{Mmt/flox}, K19 cre) in the intestine was, however, revealing (Harada et al. 1999), since it demonstrated consistency with the previously shown data and validated the genetic approach of the conditional gain-of-function mutations of β -catenin in mice.

In order to study the role of canonical Wnt signaling in pancreas development, several laboratories produced loss- and gain-of-function mutations of β -catenin in the endoderm that is committed to form pancreas (Table 1; Dessimoz et al. 2005; Murtaugh et al. 2005; Heiser et al. 2006; Rulifson et al. 2007; Wells et al. 2007). Development of the pancreas starts with two epithelial invaginations of the posterior foregut endoderm, which expand and invade the surrounding mesenchyme. There they branch and differentiate to form two lobes of the mature pancreas (for review, see Wells and Melton 1999). Gut rotation brings these dorsal and ventral lobes to a close

Grigoryan et al.

proximity, where they undergo partial fusion. The mature pancreas consists of two functionally different compartments: endocrine and exocrine. The exocrine compartment occupies most of the pancreatic mass and consists of acinar cells, which produce digestive enzymes. These enzymes are directed to the intestine through a branched network of ducts. The endocrine compartment is localized to the islets of Langerhans and is comprised of several hormone-producing cell types, the most prominent being β cells, producing insulin; α cells, producing glucagon; δ cells, producing somatostatin; PP cells, producing pancreatic polypeptide; and ϵ cells, producing Ghrelin (for review, see Wells and Melton 1999).

β -Catenin gain-of-function mutations in mice demonstrated that canonical Wnt signaling plays a crucial role in the regulation of pancreatic growth in temporal and spatial manners. By characterizing the two available Pdx1 cre lines, Heiser et al. (2006) showed that early β -catenin gain-of-function mutations, introduced using an early Pdx1 cre (expression starting E11.5), resulted in pancreatic agenesis and reduction in both endocrine and exocrine compartments (Table 1). In contrast, when β -catenin gain-of-function mutations were introduced using the late Pdx1 cre (expression starting E12.5), the pancreas was enlarged by >50% due to an increase in the proliferation of the exocrine compartment, whereas the endocrine compartment was not affected. Similar results were observed using an inducible Pdx1 creER line (Heiser et al. 2006). A role for another member of the Wnt signaling cascade, GSK3, downstream from Wnt and insulin signaling has been shown (McManus et al. 2005). These results indicate that canonical Wnt signaling plays various roles at different stages of pancreas development. In addition, it has also been shown that β -catenin acts upstream of Fgf10 and Hedgehog signaling in mediating pancreatic hypoplasia and cyst formation (Heiser et al. 2006). The characterization of the two Pdx1 cre transgenic models might, in part, explain the previously observed differences in pancreatic phenotypes following β -catenin loss-of-function mutations. β -Catenin loss-of-function mutants using early Pdx1 cre demonstrated pancreatic hypoplasia associated with a dramatic reduction of the exocrine tissue (Murtaugh et al. 2005; Wells et al. 2007). Expression of Ptf1a, an essential gene in acinar development, was lost in the β -catenin loss-of-function mutant mice. Similar results were obtained when loss-of-function mutations of β -catenin were introduced specifically into the acinar cell lineage, using an inducible Elastase cre^{ERT} (Murtaugh et al. 2005). Conversely, Dessimoz et al. (2005) demonstrated that loss-of-function mutations of β -catenin, using late Pdx1 cre affected the endocrine pancreas. The respective total numbers of insulin and glucagon-expressing cells dropped to about a half of their normal levels (Dessimoz et al. 2005). Gain-of-function mutations of β -catenin introduced specifically in the β -cell lineage, using rat insulin II promoter-driven cre (RIP cre), demonstrated a threefold increase in the proliferation of β cells and a 2.5-fold increase in β -cell mass (Table 1; Rulifson et al. 2007). Serum insulin levels in these mice were signifi-

cantly increased, and blood glucose concentration was reduced. The increase in β -cell proliferation was associated with an increase in expression of Wnt signaling targets cyclinD2 and Pitx2. These results suggest that Wnt signaling is required for cyclinD2- and Pitx2-mediated β -cell proliferation (Rulifson et al. 2007). Overall, these results demonstrated that canonical Wnt signaling plays a role at different stages of pancreas development. In early stages, it affects overall pancreas development, whereas in late stages, it is required for exocrine and endocrine cell proliferation. In view of a recent discovery that pancreas size is limited by the size of the progenitor cell pool that is specified during early pancreatic development (Stanger et al. 2007), it would be important to investigate what impact Wnt signaling has on this progenitor pool.

Wnt/ β -catenin signaling in the CNS

Various signaling systems play crucial roles in the development of different cell types and tissues during the formation of the CNS, and here, too, Wnt signaling has been found to be essential (Table 1). Neural progenitor cells are located in defined areas of the CNS; the precursors proliferate to renew the progenitor pool, while giving rise to postmitotic neuronal cells that move out of the progenitor areas (Temple 2001; Zhao et al. 2008). The progenitors receive information that allocates positional identity in the early stages of nervous system development. This positional identity will eventually determine whether a progenitor will become a glial or a neuronal cell and if the latter, which type it becomes. Positional identity is transmitted via signaling systems, which work along the A–P and D–V axes of the neural tube. These signaling systems also control progenitor proliferation, the size of the tissue domains, and the number of neurons generated by the precursors. Wnt signaling plays important roles at different stages of this developmental program, as demonstrated by the complex expression patterns of Wnts and Wnt receptors, as well as by knockdown and overexpression studies in *Xenopus*, zebrafish, chick, and mice (for instance, see Capecchi 1990; McMahan and Bradley 1990; Muroyama et al. 2002; Bonner et al. 2008). Wnt signals can direct cell fates in a concentration-dependent manner, thus acting as morphogens (for review, see Yamaguchi 2001). Noteworthy are early studies that demonstrated the expression of Wnts in various patterning centers of the nervous system, for instance, in the mid-hindbrain organizer or in the telencephalon. These signals are now known to control proliferation and cell fate specification of neuronal progenitors in these regions. The functions have usually been recognized and analyzed in mutants that lack genes encoding Wnt ligands or receptors (McMahan et al. 1992; Sur and Rubenstein 2005).

Loss- and gain-of-function mutations of β -catenin have been used to systematically investigate the role of Wnt signaling in the spinal cord, using Brain 4 (Brn4) cre (Table 1; Zechner et al. 2003, 2007). The most pronounced effect of these mutations was changes in the

size of the spinal cord. It is significantly smaller in loss-of-function mutants (Fig. 4a,b) and significantly enlarged in the β -catenin gain-of-function mutants (Fig. 4a,c). Similar effects were seen in the ventricular zones occupied by progenitor cells, which were reduced in the loss-of-function and were enlarged in the gain-of-function mutant mice (Fig. 4a–c, marked green, with anti-Nestin antibodies). Predictably, lower numbers of progenitors were accompanied by higher numbers of differentiated neurons, and an increase in progenitors was correlated with lower numbers of differentiated cells (Fig. 4a–c, red,

marked by anti Tubulin J1 antibodies). The change in size of the spinal cord is the result of a shift in the balance between precursor renewal and differentiation: Loss of β -catenin increases the probability that a precursor will undergo neuronal differentiation instead of self-renewal, whereas β -catenin gain-of-function mutation has the converse effect (Zechner et al. 2003). These data are in line with earlier reports that relied on the overexpression of activated β -catenin in the chick spinal cord by electroporation, or transgenic expression of Nestin- Δ N90 β -catenin in mice: Overexpression resulted in in-

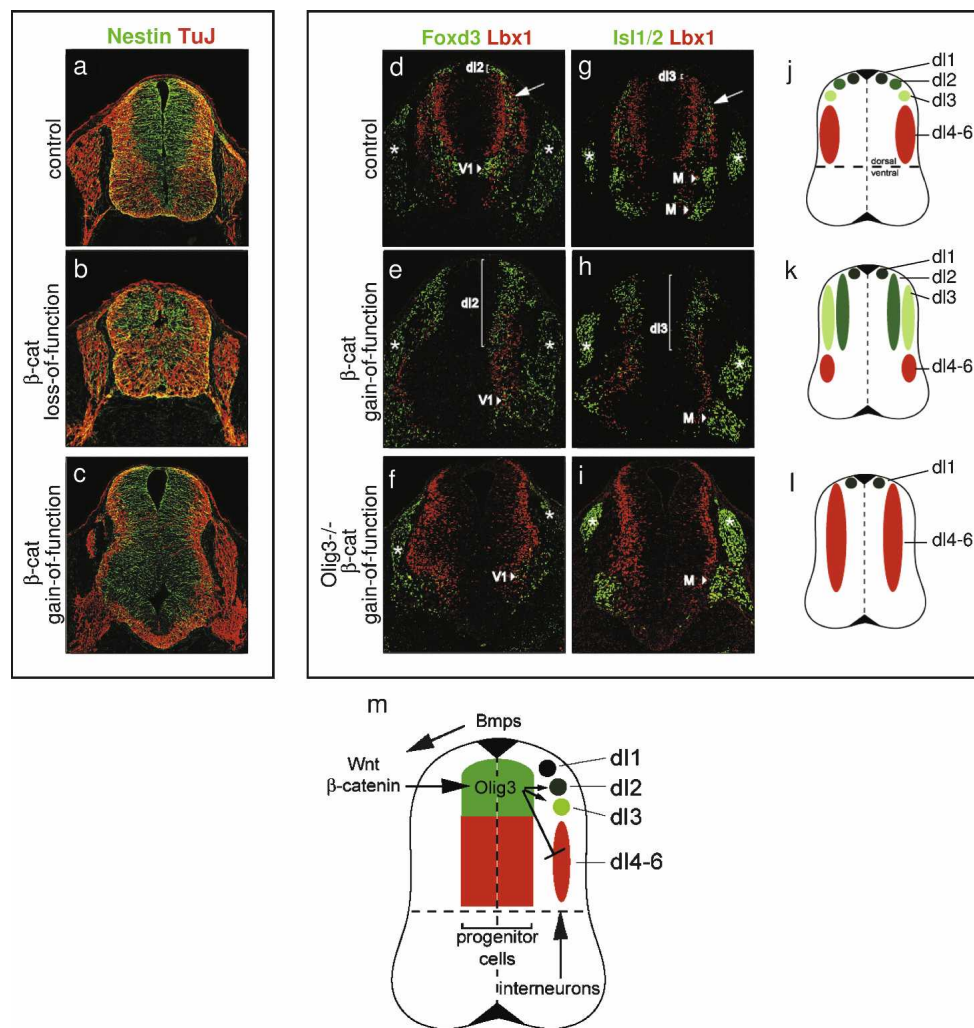


Figure 4. Wnt/ β -catenin signaling in the spinal cord. (a–c) Changes in the size of the neural progenitor pool in β -catenin mutants. At E11.5, sizes of spinal cords and the neural progenitor pool are reduced in β -catenin loss-of-function mutants (a,b) and increased in β -catenin gain-of-function mutants, as shown by Nestin-specific antibodies in green (a,c). Accordingly, the mantle zones are increased in the loss-of-function mutants (a,b) and decreased in gain-of-function mutants (a,c), as shown by Tubulin J1-specific antibodies in red. (d–m) Changes in neuronal specification in β -catenin mutants. Differentiated neurons are shown by antibody staining against Foxd3, dl2 (darker green), Isl1/2, dl3 (light green), and Lbx1, dl4–6 (red). Arrowheads label motor neurons (M) and V1 interneurons (V1) in the ventral spinal cord, and asterisks label the dorsal root ganglia. Progenitor domains that generate dl2 and dl3 neurons are expanded in β -catenin gain-of-function mutants (cf. d,g,j and e,h,k, labeled with vertical brackets). In compound mutants that carry a gain-of-function mutation of β -catenin and loss-of-function mutations of Olig3, only Lbx1+ but not Foxd3+ and Isl1/2+ neurons are generated in the dorsal spinal cord (f,i,l), indicating requirement of Olig3 expression for dl2 and dl3 neuron generation. (m) In the neuronal progenitor cells, Wnt/ β -catenin acts downstream from Bmp signaling to promote specification of dl2/3 neuronal lineages and restrict expansion of dl4–6 lineages via the patterning gene Olig3. Reprinted with permission from Zechner et al. (2003, 2007) with permission from Elsevier.

Grigoryan et al.

creased precursor proliferation and a decrease in the neuronal precursor pool, whereas loss-of-function had the opposite effect (Chenn and Walsh 2002; Megason and McMahon 2002).

In parallel, Wnt/ β -catenin signals pattern the dorsal spinal cord. Six distinct progenitor domains can be distinguished in the dorsal spinal cord based on the expression of different combinations of basic helix–loop–helix (bHLH) transcription factors, like Olig3 (Müller et al. 2005). From these progenitor domains, six types of post-mitotic neurons, dI1–dI6, distinguishable by the expression of homeobox transcription factors like Foxd3 (expressed in dI2, Isl1/2 in dI3, and Lbx1 in dI4–6), emerge in the lateral mantle zone. In β -catenin gain-of-function mutant mice, the expression of the patterning gene Olig3 was massively expanded in dorsal precursors, while in the β -catenin loss-of-function mutant mice, the Olig3 expression domain was extinguished (Zechner et al. 2007). These changes in the progenitor zone were accompanied by changes in specification of neurons: Supernumerary and ectopic Foxd3⁺ (dI2) and Isl1/2+ (dI3) neurons were generated in the gain-of-function mutant (Fig. 4e,h,k; Zechner et al. 2007). In line with this finding, earlier ablation of Wnt1/3a in mice had resulted in a reduction of the numbers of dI2 and dI3 neurons (Muroyama et al. 2002). Previous genetic experiments had shown that Olig3 in the progenitor domain is essential for specification of dI2 and dI3 neurons (Müller et al. 2005). Therefore, we generated compound mutant mice that carry a gain-of-function mutation in β -catenin and a loss-of-function mutation in Olig3. The proliferative response in the progenitor zone was still observed, while the β -catenin-dependent specification of supernumerary dI2 and dI3 neurons was blocked in the compound mutant mice (Fig. 4f,i,l; Zechner et al. 2007). Another finding was that Bmp signals act upstream of Wnt/ β -catenin signals in the control of Olig3 expression and the specification of dorsal interneurons (Zechner et al. 2007). Other studies in the mouse and chick had suggested that Wnt signaling acts downstream from Bmp signaling in the expansion of progenitor population, rather than in patterning in the dorsal spinal cord (Chesnutt et al. 2004; Ille et al. 2007). Recent data obtained from zebrafish experiments, consistent with findings in the mouse, revealed a dual role of Wnt signaling both in proliferation and in patterning. In the zebrafish, the two activities of the Wnt signaling system are mediated by distinct mechanisms: Proliferation is controlled by a Wnt signal via Tcf3, and patterning of the dorsal spinal cord is controlled by a Wnt signal that is mediated by Tcf7; this is Tcf4 in the mouse (Bonner et al. 2008).

Several lines of evidence indicate that Wnts also take over functions in A–P patterning of the brain and spinal cord. Several canonical and noncanonical Wnt ligands are expressed along the neural A–P axis, where they are thought to have developmental functions (Kimura et al. 2000; Perea-Gomez et al. 2001). The importance of inhibiting the Wnt pathway for brain specification in the anterior neural tube is demonstrated by genetic studies in zebrafish, *Xenopus*, and mice (Kazanskaya et al. 2000;

Kim et al. 2000; Kiecker and Niehrs 2001; Yamaguchi 2001). In mice, overexpression of Wnt8c resulted in posterior axis duplication and a loss of the anterior neural structures (Popperl et al. 1997). Moreover, loss of Dickkopf1 (Dkk1) expression led to posteriorization of the anterior neuroectoderm and loss of forebrain structure, but did not affect the spinal cord (Kimura et al. 2000; Mukhopadhyay et al. 2001; Perea-Gomez et al. 2001; del Barco Barrantes et al. 2003). Finally, recent studies demonstrated that guidance of commissural axons along the A–P axis of the spinal cord is mediated by Wnts (Lyuksyutova et al. 2003). Future studies in which β -catenin undergoes conditional loss- and gain-of-function mutations in the early stages of neural tube development could help to understand the role of canonical Wnt signaling in patterning the neural A–P axis and separate canonical from noncanonical Wnt signaling in this complex process. Loss-of-function mutations of β -catenin have also been used to study brain morphogenesis, using Nestin, D6, Emx1, and proopiomelanocortin cre lines (Table 1; Machon et al. 2003; Campos et al. 2004; Gao et al. 2007; Schuller and Rowitch 2007). These studies have demonstrated the important role of β -catenin-mediated signals in the formation of the cerebral cortex and hippocampus. Moreover, conditional ablation of β -catenin in postnatal motorneurons using human skeletal actin cre (HSA cre) revealed a role of canonical Wnt signaling in neuro-muscular junction formation (Li et al. 2008). Overall, the studies of loss- and gain-of-function mutations of β -catenin in the CNS demonstrated that Wnt signals direct differentiation, for instance, determine subtypes of sensory neurons and pattern the spinal cord. In parallel, Wnt signals allow maintenance and expansion of the corresponding progenitor pool.

Wnt/ β -catenin signaling in the neural crest

As in the CNS, canonical Wnt signaling is essential in the peripheral nervous system (PNS), where it steps in at an early stage of neural development to help direct cell migrations and patterning in the neural crest. The neural crest begins as a population of multipotent cells that arise at the border between neural and nonneural ectoderm in vertebrates. As the neural tube closes, neural crest cells delaminate dorsally in a rostro-caudal wave and migrate along specific routes throughout the embryo, generating a broad variety of neural and nonneural derivatives. These include peripheral neurons and glia, the craniofacial skeleton, connective tissue and cartilage, pigment cells, and the neuroendocrine epithelium of adrenal glands (for review, see Gammill and Bronner-Fraser 2003). The differentiation potential of neural crest cells varies along the A–P axis. Cranial neural crest cells give rise to the craniofacial skeleton and cranial ganglia. Vagal and sacral neural crest generate parasympathetic as well as enteric ganglia and contribute to the heart. Trunk neural crest cells give rise to dorsal root ganglia, the sympathetic nervous system, and chromaffin cells of the adrenal gland, as well as melanocytes. A large amount of data obtained in *Xenopus*, zebrafish, chick,

and mouse has provided evidence that the Wnt pathway is required for neural crest induction and differentiation (Saint-Jeannet et al. 1997; Chang and Hemmati-Brivanlou 1998; LaBonne and Bronner-Fraser 1998; Tamai et al. 2000; Dearnorff et al. 2001; Garcia-Castro et al. 2002; Abu-Elmagd et al. 2006). In the mouse, compound mutant embryos lacking both Wnt1 and Wnt3a show major deficits in neural crest derivatives due to a reduction in neural crest cells (Ikeya et al. 1997). Since not all neural crest derivatives were affected to a similar extent, it was proposed that Wnt1 and Wnt3a might be responsible for the generation of specific subsets of neural crest cells, but not all types (Salinas and Nusse 1992; Parr et al. 1993).

Conditional loss-of-function mutations of the β -catenin gene using Wnt1 cre, which introduces cre-mediated recombination in the dorsal neural tube and all neural crest cells, resulted in dramatic defects in neural crest derivatives (Table 1; Brault et al. 2001). Cranial bones and cartilage of cranial neural crest origin were absent. Craniofacial development requires the communication between cells derived from the neural crest and the cranial paraxial mesoderm, which generates muscle. Conditional β -catenin/Wnt1 cre mutant mice permitted an assessment of this interaction, due to the severe changes in cranial neural crest cell differentiation. Neural crest cells are dispensable for cranial muscle progenitor specification but are required for muscle patterning (Rinon et al. 2007). Furthermore, the formation of a further neural crest cell derivative, the heart outflow tract, is also affected in the conditional Wnt1 cre/ β -catenin mutant, which is discussed in the next section (Kioussi et al. 2002). In addition, melanocytes did not form, as demonstrated by the absence of the expression of melanocyte-specific genes, such as *Mitf*, a bHLH transcription factor essential for melanoblast specification (Dorsky et al. 2000; Takeda et al. 2000; Hari et al. 2002; Larue et al. 2003; Saito et al. 2003). Neuronal derivatives of the neural crest also require canonical Wnt signals for differentiation (Hari et al. 2002). Neural crest cells in the loss-of-function mutant mice failed to differentiate into the sensory neurons (Hari et al. 2002; Lee et al. 2004). Conditional gain-of-function mutants of β -catenin, again using Wnt1 cre, exhibited a converse phenotype (Lee et al. 2004): Neurogenin-2-expressing sensory neurons were found ectopically in the anterior part of the embryo. Thus, activation of canonical Wnt signaling resulted in the expansion of the sensory neuronal lineage at the expense of other neural crest derivatives, indicating that neural crest cells are instructed by Wnt/ β -catenin signals to assume a sensory neuronal fate (Lee et al. 2004). Moreover, the neural crest of such mutants was unable to generate enteric ganglia and Schwann cells that associate with peripheral axons.

These data on the use of loss- and gain-of-function β -catenin mutants in mice demonstrate that β -catenin controls many aspects of neural crest cell development, including survival, progenitor cell expansion, and important lineage decisions. These data, together with experiments in *Xenopus*, zebrafish, chick, and mouse, suggest

that Wnt signaling acts at several stages of neural crest development. At an early stage, Wnt appears to instruct neuronal progenitors that migrate to the dorsal spinal cord to assume a neural crest cell fate (Saint-Jeannet et al. 1997; Lewis et al. 2004), whereas at later stages, Wnt induces expansion and lineage decisions of the neural crest cells. These functions of Wnt/ β -catenin in neural crest development seem to occur independently of its general role in A–P patterning (for review, see Raible and Ragland 2005).

Wnt/ β -catenin signaling in cardiogenesis

Investigations of heart development and homeostasis in mice are prime examples of cases in which many different types of conditional loss- and gain-of-function mutations have been used successfully to expose the roles of Wnt/ β -catenin signaling (Table 1). The vertebrate heart originates from three separate progenitor cell lineages, the mesoderm-derived first and second heart fields, and the neural crest (see Fig. 5a; Buckingham et al. 2005; Srivastava 2006). Initially, the cells of first and second heart fields (the crescent) migrate toward the midline to form a linear cardiac tube. Cells of the first heart field then contribute to the future left ventricle. Cells of the second heart field proliferate and migrate and direct the rightward looping of the cardiac tube, thereby contributing to the future right ventricle, and inflow and outflow tracts (Buckingham et al. 2005). Neural crest-derived precursors contribute to the complex organization of the heart outflow tract. A role of Wnt/ β -catenin signaling in heart development has been postulated previously, based on expression profiles of Wnt pathway members, as well as on explant studies in animal models, and in cell culture (for example, see Frasch 1995; Marvin et al. 2001; Schneider and Mercola 2001; Tzahor and Lassar 2001; Nakamura et al. 2003; Foley and Mercola 2005; Eisenberg and Eisenberg 2006).

Conditional ablation of the β -catenin gene in mesodermal precursors of first and second heart field progenitors during development did not affect the first heart field, but resulted in defects of the second heart field (Klaus et al. 2007). This work used mesoderm posterior 1 (*MesP1*) promoter-driven cre, the earliest available cre that targets heart lineages (Saga et al. 2000; Klaus et al. 2007). The linear heart tube was correctly formed, while cardiac looping, proper outflow tract, and right ventricle formation were prevented (Fig. 5b,c). The self-renewal of *Islet1*-expressing progenitor cells, which contribute to second heart field derivatives, was impaired. In contrast, constitutive activation of β -catenin again using *MesP1* cre resulted in disruption of the linear heart tube (Fig. 5, cf. b and d) and in expansion of *Islet1* progenitors. The loss- and gain-of-function mutations of β -catenin therefore suggest that absence of Wnt/ β -catenin signaling allows first heart field generation, and that the generation of the second heart field may depend on the correct amount and timing of Wnt/ β -catenin signaling (Klaus et al. 2007). Consistent with these data, similar phenotypes were produced by removing competence to respond to

Grigoryan et al.

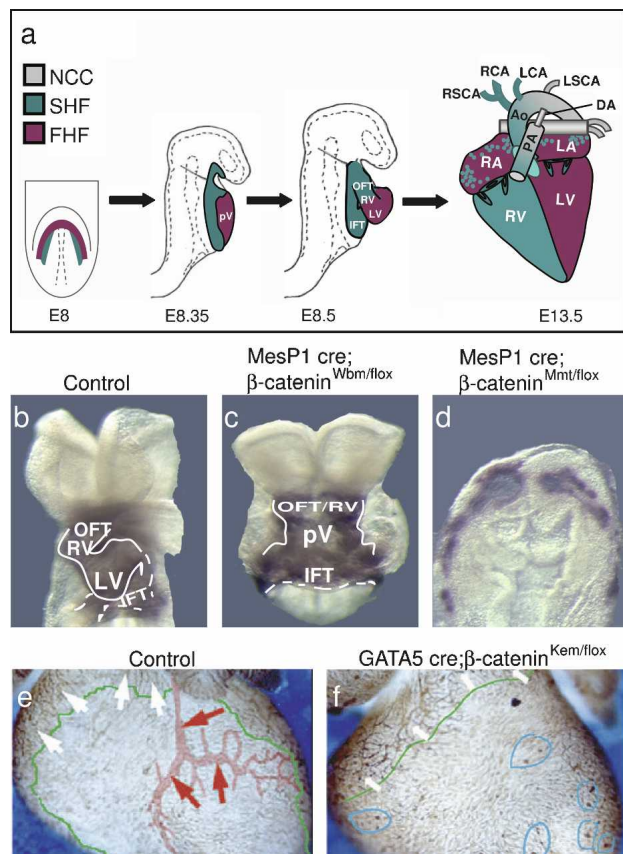


Figure 5. Wnt/ β -catenin signaling during cardiogenesis. (a) Schemes of mouse embryos at different stages of embryonic development show the location and contribution of first (red) and second (green) heart field cells at cardiac crescent (frontal view), linear heart tube, and looping stages (lateral views). Following rightward looping of the linear heart tube, neural crest cells migrate into the outflow tract and take part in septation and remodeling of the outflow tract, resulting in a multichambered heart at E13.5. (b–d) Conditional ablation and activation of β -catenin using *MesP1*-driven cre shows the loss of cardiac looping in *MesP1 cre; β-catenin^{lox/lox}* mice (b,c), and the disruption of cardiac tube formation in *MesP1 cre; β-catenin^{loxEx3/+}* mice (b,d) as indicated by whole-mount in situ hybridization for *Nkx2.5* and *MLC2a* at E8.5. (e,f) Conditional ablation of β -catenin in the proepicardial lineage (*Gata5 cre*) resulted in the absence of coronary arterial vasculature as indicated by PECAM 1 staining at E18.5 (cf. red arrows in e with f). (Ao) Aorta; (DA) ductus arteriosus; (FHF) first heart field; (IFT) inflow tract; (LV) left ventricle; (NCC) neural crest; (OFT) outflow tract; (PA) pulmonary artery; (pV) primitive ventricle; (RV) right ventricle; (RA and LA) right and left atrium; (RCA) right carotid artery; (LCA) left carotid artery; (RSCA) right subclavian artery; (LSCA) left subclavian artery; (SHF) second heart field. b–d are reprinted with permission from Klaus et al. (2007); © 2007 National Academy of Sciences, U.S.A. e and f are reprinted with permission from Zamora et al. (2007); © 2007 National Academy of Sciences, U.S.A.

Wnt signaling at later stages of heart development. This was accomplished through the ablation of β -catenin in differentiating cardiac progenitors, using *Nkx2.5 cre*, or in the second heart field using *Islet1-cre* or *Mef2c-AHF*

cre (Table 1). The phenotypes included defects in second heart field derivatives, partially due to prevention of proper *Islet1* progenitor expansion. Conversely, sustained activation of β -catenin in these cell lineages strongly induced self-renewal of second heart field cells and an expansion of their derivatives (Ai et al. 2007; Kwon et al. 2007; Lin et al. 2007; Qyang et al. 2007). Additionally, gain- and loss-of-function mutations of β -catenin in myocardial and smooth muscle cells using smooth muscle-22 α cre indicated that Wnt signaling may act upstream of FGF signaling in the regulation of proliferation of *Islet1*-expressing second heart field cells (Cohen et al. 2007).

Genetic manipulations in the mouse have also shown that Wnt/ β -catenin signaling contributes to the remodeling of both cardiac blood vessels and the neural crest-derived heart outflow tract (Table 1). A loss-of-function mutation of β -catenin in the heart's neural crest cells, using *Wnt1 cre*, affected outflow tract remodeling mediated by transcriptional regulation and activation of *Pitx2*, which, in turn, was shown to be required for the proliferation of neural crest cells that contribute to the outflow tract (Kioussi et al. 2002; see also previous section). Furthermore, loss-of-function mutations of β -catenin, using endothelial-specific *Tie2 cre*, showed that endocardial cells require β -catenin's transcriptional activity for endothelial–mesenchymal transition during endocardial cushion formation (Liebner et al. 2004). The role of the Wnt pathway in cardiac vessel formation has been also demonstrated by loss-of-function mutations of β -catenin in epicardium-derived cells, using *Gata5 cre*. Mutant embryos showed both impaired coronary artery formation and the differentiation of epicardium-derived mesenchymal cells into the smooth muscle lineage (Fig. 5e,f; Zamora et al. 2007). Strikingly, conditional ablation of the β -catenin gene in the mouse embryonic endoderm, using cytochrome 19 cre, caused cell fate changes from endoderm to cardiac mesoderm, which resulted in ectopic formation of multiple hearts (Lickert et al. 2002). The investigators conclude that canonical Wnt signaling function in cardiogenesis is, at least in part, due to a cell autonomous role of Wnt signaling in the early endoderm. In the adult heart, loss-of-function mutations of β -catenin, using two inducible α -myosin heavy chain promoter-driven cre lines, resulted in cardiac hypertrophy (Chen et al. 2006; Baurand et al. 2007; Zhou et al. 2007). It has been shown that the pathological, stress-induced growth of cardiomyocytes is dependent on Wnt/ β -catenin target gene repression rather than on its adhesive function. Similarly, cardiac-specific haploinsufficiency of β -catenin by heterozygous loss-of-function mutations using noninducible α -myosin heavy-chain cre, attenuated cardiac hypertrophy following transverse aortic constriction (Qu et al. 2007).

In conclusion, genetic ablation and activation of the β -catenin gene in different cardiac cell lineages at different stages of heart development and in the adult (Table 1) provided a complex picture of the temporally and tissue-specific roles of Wnt/ β -catenin signaling during specification, self-renewal, and differentiation of various popu-

lations of cardiac progenitor cells. Wnt/ β -catenin signaling is thus required to maintain and expand cardiac precursor lineages, and it needs to be repressed in order to promote differentiation into cardiomyocytes or smooth muscle cells. How canonical Wnt signaling crosstalks with other developmental signaling pathways and how it controls the transcriptional networks that are essential for heart formation remain to be established.

Wnt/ β -catenin signaling in sensory placode derivatives

Recent studies show that Wnt signaling plays crucial developmental roles in the sensory organs. Sensory organs such as the eye, inner ear, and taste buds share a common developmental origin, the interplay of placode and (neuro) ectoderm tissues (Table 1; for review, see Bailey and Streit 2006).

The taste buds of the tongue consist of two compartments, the tongue epithelium in the shape of papillae and the endings of the gustatory nerves, which are of placodal origin, that form clusters of neuronal cells in the center of those papillae. Several types of tongue papillae can be distinguished—fungiform, foliate, and circumvalate—which are organized in a stereotypic pattern on the anterior two-thirds of the dorsal surface of the tongue. Wnt signaling's role in taste bud development has only recently become evident, when loss- and gain-of-function mutation of β -catenin was examined in the tongue epithelium, using K14 cre (Table 1). Moreover, the activation of the Wnt pathway with the GSK3-inhibitor LiCl in tongue cultures led to an increase in the number of taste placodes (Iwatsuki et al. 2007; Liu et al. 2007). Ablation of the β -catenin gene in the tongue epithelium, using K14 cre (the same model used to study skin), blocked fungiform taste bud development, as demonstrated by the loss of expression of Shh and Bmp4, indicating that the canonical Wnt pathway is required for the early induction of this developmental process. Overexpression of the Wnt inhibitor Dkk1 under the K5 promoter and ablation of Wnt10b or Lef1 also resulted in severe reduction or complete loss of all fungiform papillae (Iwatsuki et al. 2007; Liu et al. 2007). In contrast, gain-of-function mutations of β -catenin in the tongue epithelium, again using K14 cre, resulted in the opposite phenotype, a higher placode density and ectopic placode formation (Liu et al. 2007). It has also been shown that the defective taste bud epithelium indirectly affects taste bud innervation. In later stages, Wnt signaling appears to be required for maintaining placode homeostasis. Activation of the Wnt pathway promotes placode formation, whereas in the absence of the Wnt pathway, cells lose placode identity and, instead, adopt an interplacodal fate (Liu et al. 2007). Candidate factors that act downstream from canonical Wnt signaling in placode formation are Sox2 and Shh. Indeed, it has been shown in Sox2 hypomorphic embryos that decrease of Sox2 leads to a reduction in fungiform placode numbers. In contrast, overexpression of Sox2 increases fungiform papillae at the expense of filiform papillae development (Okubo et al. 2006).

Recent studies have implicated canonical Wnt signaling in development of the lens and retina. In eye development, mutual inductive signals between optic vesicles and the overlying placodal surface ectoderm promote the thickening of the ectoderm to form the lens placode. Lens placode and optic vesicle further invaginate, resulting in formation of both the lens vesicle and the double-layered optic cup. The inner layer of the optic cup gives rise to the neural retina, while the outer layer of the optic cup forms the retinal pigmented epithelium (RPE). Various Wnt ligands, Frizzled receptors, and secreted Wnt antagonists, such as Dkk and sFPRs, are expressed in complex patterns during mammalian lens and retina development (Liu et al. 2003; Stump et al. 2003; Ang et al. 2004; Chen et al. 2004). Studies of various Wnt reporter mice have permitted the mapping of canonical Wnt signaling during the eye development (Miller et al. 2006; Kreslova et al. 2007). Conditional loss-of-function mutations of β -catenin in the lens placode and surrounding head surface ectoderm were introduced by various cre lines, among those the lens cre, LR cre, and Nestin cre (Table 1). In the lens cre, cre expression is driven by the ocular ectoderm-specific enhancer of the Pax6 gene (Ashery-Padan et al. 2000; Smith et al. 2005). LR-cre is expressed in the developing lens and partially, retina. Nestin cre is expressed in the lens epithelium later in development and was used to study lens development at subsequent stages (Kreslova et al. 2007). Mutant β -catenin embryos showed abnormal eye and lens morphology. Interestingly, loss of β -catenin in the periocular area resulted in formation of ectopic lentoid bodies that expressed β -crystallin and Prox1. Introduction of the gain-of-function mutation of β -catenin, again using lens cre, led to a loss of lens structure and lens-specific marker expression, and to an up-regulation of neural-specific markers, such as β -tubulin (Miller et al. 2006). These data suggest that inhibition of canonical Wnt signaling within the lens placode and its activation in the periocular ectoderm are required for the proper lens formation and patterning (Smith et al. 2005). Interestingly, defects in retina development were also observed in the LR cre mutants, since LR cre is mosaically expressed in the retina (Kreslova et al. 2007). A role of canonical Wnt signaling in retinal development, patterning, and regeneration had been previously suggested (Liu et al. 2003; Ouchi et al. 2005; Miller et al. 2006; Osakada et al. 2007).

The inner ear is a composite sensory structure, characteristic for vertebrates, that is responsible for detection of sound, balance, and acceleration. During development, the inner ear originates from a region of the thickened surface ectoderm, the so-called otic placode. Induction of the otic placode is a multistep process that requires signals from the adjacent posterior hindbrain and the cranial paraxial mesoderm (for review, see Streit 2001). A current model proposes two steps in otic placode induction: First, a "preotic placode" separates from a common region of competence for all the craniofacial placodes; during the second step, this preotic placode differentiates into the otic placode and the nonotic sur-

Grigoryan et al.

rounding ectoderm (Ohyama et al. 2006). Genetic studies in *Xenopus*, zebrafish, chick, and mouse have implicated various signaling pathways to be involved in otic placode induction and patterning (Streit 2001; Mohamed et al. 2004; Ohyama et al. 2006; for review, see Ohyama et al. 2007). To elucidate the role of Wnt signaling during otic placode formation, Ohyama et al. (2006) induced loss- and gain-of-function mutations of β -catenin with Pax2 cre (Table 1). The otic vesicles of loss-of-function embryos showed a severely reduced size, accompanied by increased size of the surrounding cranial ectoderm. Reduced proliferation, increased apoptosis, and a disruption of cell-cell adhesion was detected at the late, but not at the early stages of otic development. In the mutant embryos, expansion of the ectoderm occurred at the expense of the otic placode tissue. An opposite phenotype was observed in β -catenin gain-of-function mutations: Mutant embryos showed an expansion of the otic placode, overexpression of otic markers in ectopic locations, and a reduction of nonplacodal ectoderm. The expansion of the otic placode, which expressed markers *Dlx5*, *Gbx2*, and *Msx1*, was due to a higher proliferation rate (Ohyama et al. 2006). These results suggest that canonical Wnt signaling plays a crucial role in mediating decisions of the Pax2-expressing preotic placode between placodal and epidermal fates. So cells within the preotic field, which receive a high Wnt signal, differentiate into otic placode, whereas cells that receive no or little Wnt signal differentiate into epidermis. During later stages of otic development, canonical Wnt signaling plays a role in D-V patterning of the otocyst (Ohyama et al. 2006). β -Catenin gain-of-function mutants showed a large expansion of dorsal markers at the expense of the ventral markers. These data are consistent with the previous experiments, which demonstrated that a balance between dorsal Wnt and ventral Shh signals is essential in order to promote the regional identity within the otic epithelium and to coordinate the morphogenesis of the vestibular (dorsal) and auditory (ventral) organs (Riccomagno et al. 2005). It remains to be studied to what extent the mechanism of Wnt interaction with other pathways is involved in the induction and patterning of the otic placode—e.g., with FGF—as well as a possible role of canonical Wnt signaling in the late ear and vestibular development. Wnt may interact with FGF via Sox2 (Ohyama et al. 2007).

The pituitary gland is a neuroendocrine organ, which controls a range of vital functions of vertebrates, including body growth, metabolism, lactation in the mammary gland, reproduction, stress response, and aging (for review, see Zhu and Rosenfeld 2004). During early development, two embryonic tissues contribute to pituitary gland formation: the oral ectoderm, which gives rise to the anterior and intermediate lobes, and the pituitary placode, which contributes to the so-called Rathke's pouch (Zhu and Rosenfeld 2004). The posterior pituitary is of neural origin and consists of axons of neurosecretory neurons (Scully and Rosenfeld 2002). The anterior and intermediate lobes of the pituitary gland contain six types of hormone-secreting cells: corticotropes (which

secrete adrenocorticotrophic hormone), thyrotropes (secreting thyroid-stimulating hormone), somatotropes (growth hormone), lactotropes (prolactin), gonadotropes (luteinizing hormone and follicle-stimulating hormone), and melanotropes (melanocyte-stimulating hormone). Specification of the somatotrope, lactotrope, and thyrotrope lineages depends on the essential POU-class homeodomain transcription factor Pit1 (Olson et al. 2006). Conditional loss-of-function mutations of the β -catenin gene in pituitary progenitors, using Pitx1 cre, resulted in a complete loss of Pit1 lineage derivatives and in the loss of expression of Pit1 itself (Olson et al. 2006). Conditional gain-of-function mutations of β -catenin, again using Pitx1 cre, did not block initial organ commitment at E9.5, but at later developmental stages, the whole gland was absent. In contrast, in loss-of-function mutations of β -catenin using Pit1 cre, the somatotrope, lactotrope, and thyrotrope lineages were still formed, which indicates that Wnt/ β -catenin signaling is not required for their maintenance (Olson et al. 2006). In this study, a novel mechanism of Wnt/ β -catenin signaling was identified: β -catenin directly associates with the paired-like homeodomain transcription factor Prophet of Pit1 (Prop1) to activate Pit1 expression. This has been confirmed in Prop1^{-/-} mice, which, indeed, display absence of the three pituitary lineages. Previous work demonstrated that earlier in pituitary development, Wnt/ β -catenin signaling controls proliferation of the pituitary progenitors by directly regulating expression of the essential gene Pitx2 (Fig. 6; Kiousi et al. 2002). Activation of Wnt signaling leads to the binding of β -catenin and other coactivator complexes to the Pitx2 promoter (Fig.

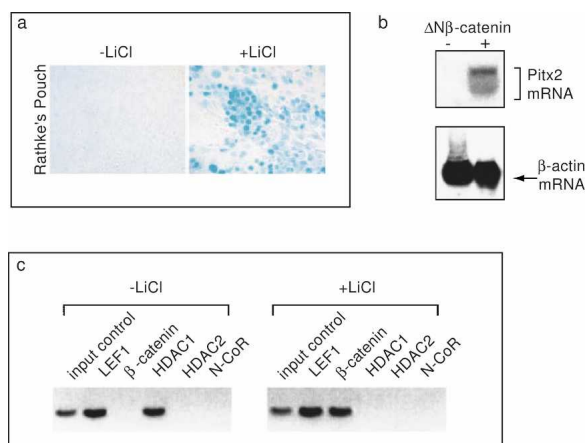


Figure 6. Wnt/ β -catenin pathway directly induces Pitx2 expression in the pituitary gland. (a) LiCl addition to the primary cell culture prepared from E10.5 pituitary glands leads to activation of the Pitx2-driven LacZ expression. (b) Overexpression of the constitutively active β -catenin (Δ N- β -catenin) in the α T₃-1 pituitary cell line leads to activation of Pitx2 gene expression. (c) Chromatin immunoprecipitation analysis demonstrated that in the LiCl-stimulated cells, β -catenin binds to the Pitx2 promoter together with LEF1, whereas in the LiCl-unstimulated cells, LEF1 binds to the Pitx2 promoter along with HDAC1. Reprinted from Kiousi et al. (2002) with permission from Elsevier.

6c), which then induces expression of the genes that control the cell cycle, such as cyclin D2. Thus, in the pituitary gland, canonical Wnt signaling acts at different developmental stages through different mechanisms: Tcf/Lef-dependent and Prop1-dependent. Additionally, in the complex with Prop1, β -catenin can act as a binary switch, activating Pit1 and simultaneously inhibiting another paired-like homeodomain protein, Hesx1 (Olson et al. 2006). Among Wnt ligands that act upstream of β -catenin in pituitary gland development, Wnt4 and Wnt5a are possible candidates. However, single as well as the double knockout of the genes encoding these ligands did not induce a dramatic phenotype (Treier et al. 1998; Cha et al. 2004). Further mechanisms of canonical Wnt signaling upstream of β -catenin remain to be elucidated.

In conclusion, Wnt signaling plays diverse roles in sensory placode derivatives. In early stages of taste bud development, Wnt signaling is required to induce taste papilla formation, whereas in the later stages, it plays a role in taste bud maintenance. In contrast, during lens and otic placode development, Wnt signaling is not required for the initial placode induction. In the lens, Wnt activity needs to be repressed, whereas in the surrounding ectoderm, it needs to be activated in order to restrict the lens-forming field. In the otic placode, Wnt signaling is required at later stages to maintain placode identity and D–V patterning. In the pituitary, different mechanisms of Wnt function could be identified: On the one hand, Wnt signaling regulates early pituitary progenitor proliferation via Tcf/Lef-dependent regulation of Pitx2, and on the other hand, regulates specification of the Pit1-expressing cells lineages via a Prop1-dependent mechanism.

Wnt/ β -catenin signaling in somitogenesis

Somites originate from presomitic mesoderm during the process of body axis elongation of vertebrate embryos. Somites are transient structures that, later in development, give rise to bones, joints, muscles, and connective tissue of the axial skeleton (Saga and Takeda 2001). In all vertebrates, somites are generated sequentially along the A–P axis at fixed time intervals (every 2 h in the mouse). Such a mechanism requires strict spatial and temporal regulation, which is accomplished by a rhythmic molecular mechanism, the so-called somitic clock. The somitic clock consists of a gradient of signaling factors such as Fgf8 and Wnt3a, and an oscillator. The latter can be visualized by an oscillating expression of genes along the presomitic mesoderm. The oscillation wave sets in at the caudal end of the embryo, where presomitic mesoderm cells are formed. As the embryo grows caudally, cells move out of the gradient of the signaling factors and start to form a segment according to positional information provided by the oscillating genes. Meanwhile, the embryo maintains a constant amount of unsegmented mesoderm by proliferation at the caudal tip. Since the molecular oscillating clock in combination with the morphogen gradient defines the regular boundary positions between the somites, this model of somitogenesis is often referred to as the “clock and gradient model”

(Saga and Takeda 2001; Aulehla and Herrmann 2004; Giudicelli and Lewis 2004). The role of Wnt signaling in somite boundary formation was initially established by experimental up-regulation or down-regulation of Wnt3a signals in the mouse presomitic mesoderm, which led to the formation of smaller or larger somites, respectively. These experiments also demonstrated oscillations of Wnt target genes such as Axin2 (a negative feedback regulator of Wnt signaling) (Lustig et al. 2002), and suggested a role of Wnt3a/ β -catenin genetically upstream of the Notch signaling pathway (Aulehla et al. 2003).

Loss- and gain-of-function mutations of β -catenin were recently used to study the complex role of canonical Wnt signaling in somitogenesis. Loss-of-function mutations of β -catenin were introduced into paraxial mesoderm precursors, using Brachyury cre (Table 1; Aulehla et al. 2008; Dunty et al. 2008). Mutant embryos displayed severe posterior axis truncation with very few abnormally formed somites, whereas the anterior paraxial mesoderm was thickened and unsegmented. Posterior presomitic mesoderm markers such as Brachyury, Tbx6, and Fgf8 were absent (Fig. 7a,b). Moreover, expression of Mesp2, an essential gene in somite boundary formation, was lost or mislocalized in β -catenin loss-of-function mutants (Fig. 7d,e). The expression of Wnt- and Notch-associated oscillating genes such as Axin2, lunatic fringe, and Hes7 was extinguished in loss-of-function mutants. In contrast, when gain-of-function mutations of β -catenin were introduced using the same cre, mutant embryos showed delayed and defective formation of somites and an expansion of presomitic mesoderm. No difference in proliferation was observed. The expression of Wnt target genes involved in the presomitic mesoderm patterning such as Tbx6, mesogenin1, and Dll1 was up-regulated and shifted anteriorly (Fig. 7a,c). Expression of Mesp2 was shifted anteriorly and appeared in multiple stripes (Fig. 7d,f). Surprisingly, the constitutive activation of β -catenin did not block expression of Wnt- or Notch-regulated oscillating genes, as demonstrated,

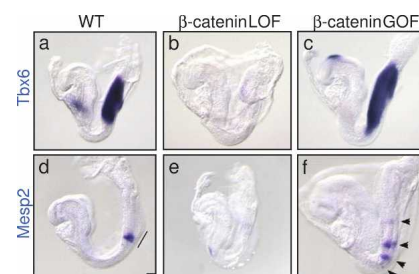


Figure 7. Wnt/ β -catenin signaling in somitogenesis. (a,b) Conditional loss-of-function mutations of β -catenin result in anterior mesoderm thickening, posterior truncation and prevention of somitogenesis. (a,b) Tbx6, a marker for posterior presomitic mesoderm, is lost in loss-of-function mutants. Conditional gain-of-function mutations of β -catenin result in posterior mesoderm expansion shown by expanded Tbx6 expression (a,c). (d–f) Expression of Mesp2 is lost in loss-of-function mutants (d,e), and appears in multiple stripes in gain-of-function mutants (d,f). Reprinted by permission of the Company of Biologists from Dunty et al. (2008).

Grigoryan et al.

for instance, by real-time imaging using lunatic fringe as a reporter and other methods. Markers for somite polarity, such as *Tbx18* and *Uncx4.1*, continued to be expressed in both loss- and gain-of-function mutants, indicating that Wnt/ β -catenin signals are not essential to establish somite polarity (Aulehla et al. 2008). Finally, a microarray analysis identified a new somite boundary determination gene, *Ripply2*, which acts downstream from Wnt and *Mesp2* to regulate segment boundary formation (Dunty et al. 2008).

These experiments have demonstrated a crucial role for nuclear β -catenin in defining the size of the oscillatory field at the posterior tip of the embryo and assigned additional roles to Wnt/ β -catenin signals in mesoderm patterning. In these processes, β -catenin protein does not cycle, but forms a nuclear-to-cytoplasmic gradient. However, the transcriptional activity of nuclear β -catenin may be modulated by oscillating cofactors. Of note, oscillating Wnt signaling activity has recently also been demonstrated in the skin, where it is required for proper hair cycle progression (Plikus et al. 2008). The functional significance of oscillating Wnt target genes as well as the detailed mechanisms of Wnt function in these complex processes remain to be determined.

Wnt/ β -catenin signaling in bone and cartilage

Canonical Wnt signals play an essential role in skeletogenesis. The mammalian skeleton is composed of two types of tissues, bone and cartilage. Bone, in turn, is formed by two major types of cells, osteoblasts and osteoclasts. Cartilage is formed by chondrocytes. Osteoblasts and chondrocytes originate from common osteo-chondrogenic progenitor cells of mesenchymal origin. Osteoclasts are cells of hematopoietic origin that take part in resorption of the newly formed bone (for review, see Karsenty and Wagner 2002; Hartmann 2006). The role of canonical Wnt signaling at different stages of skeletogenesis has been proposed based on the expression patterns of many Wnt pathway members, as well as Wnt reporter expression in the mouse (Parr et al. 1993; Guo et al. 2004; Day et al. 2005; Hens et al. 2005; Hill et al. 2005; Hu et al. 2005).

The contribution of Wnt signaling during early skeletogenesis has recently been explored through conditional loss- and gain-of-function mutations of β -catenin (Table 1). β -Catenin loss-of-function mutations were introduced into osteo-chondrogenic progenitor cells using *Prx1* and *Dermo1* cre, and resulted in early osteoblast differentiation arrest, increased chondrogenesis, and ectopic cartilage formation (Day et al. 2005; Hill et al. 2005; Hu et al. 2005). Conversely, gain-of-function mutations of β -catenin in the osteo-chondrogenic progenitors using *Prx1* cre resulted in the arrest of chondrogenesis (Hill et al. 2005). Furthermore, β -catenin loss- and gain-of-function mutations were introduced in micromass cultures of early limb buds prepared from β -catenin^{Wbm/flox} and β -catenin^{Mmt/flox} embryos that were then infected with adenoviral cre. β -Catenin loss-of-function mutations in these micromass cultures resulted

in an increase in the expression of the chondrogenic marker *Sox9* and precocious differentiation into chondrocytes, whereas β -catenin gain-of-function mutations led to the decrease of *Sox9* expression and suppressed chondrogenesis. This indicates that Wnt/ β -catenin signaling negatively regulates differentiation of mesenchymal cells into chondrocytes. Moreover, adenoviral cre-mediated ablation of β -catenin in calvarial mesenchymal progenitor cell cultures inhibited osteoblast differentiation and allowed chondrocyte differentiation, demonstrating that Wnt/ β -catenin signaling acts cell-autonomously to promote osteo-chondrogenic progenitor cells to differentiate into the osteoblast lineage (Hill et al. 2005).

Loss- and gain-of-function mutations of β -catenin using *Collagen-1a* cre have revealed an additional function for Wnt signaling in these processes (Table 1). It plays a role in osteoblasts that have already differentiated: Overexpression of β -catenin resulted in a negative effect on bone resorption and osteoclast differentiation. Mutant mice displayed a decrease in osteoclast numbers, resulting in a dramatic increase in bone mass as well as altered tooth development (Glass et al. 2005). Osteoclast differentiation was affected in both loss- and gain-of-function mutants due to the deregulation of the osteoprotegerin gene. Osteoprotegerin was proposed to be a direct target of Wnt signaling in osteoblasts, regulated by *Tcf1* (Glass et al. 2005; Jackson et al. 2005). This was a novel finding in that β -catenin may act in a non-cell-autonomous manner (Glass et al. 2005). Similar results were observed in experiments where loss- and gain-of-function mutations of β -catenin in osteoblasts were introduced with an osteocalcin cre (Holmen et al. 2005). Recently, loss- and gain-of-function mutations of β -catenin using *Osx1* cre have been used to demonstrate that commitment within the osteoblast lineage requires strictly regulated Wnt pathway activity and a stage-specific genetic interaction between Wnt and Indian Hedgehog (*Ihh*) pathways (Hu et al. 2005; Mak et al. 2006; Rodda and McMahon 2006). Apparently, in osteoblast differentiation, β -catenin acts downstream from *Ihh* signaling, whereas in chondrocyte survival, β -catenin acts upstream of *Ihh* to inhibit chondrocyte apoptosis (Mak et al. 2006). Finally, using loss- and gain-of-function mutations of β -catenin, it has been demonstrated that direct interaction between β -catenin and *Sox9* is essential to control chondrocyte maturation (Akiyama et al. 2004). Overall, in early bone development, Wnt/ β -catenin signaling mediates lineage decisions of the osteo-chondrogenic progenitors, directing them to adopt an osteoblast fate. At later stages, Wnt signaling is required for the osteoblast maturation and, indirectly for bone resorption.

Wnt/ β -catenin signaling in organs that contain branching epithelia

Canonical Wnt signaling is essential in many aspects of tissue branching in organs like kidney, lungs, and mammary gland (Table 1). The development of the mammalian metanephric kidney starts when the nephric (Wolffian) duct of mesodermal origin invaginates to form the

ureteric bud (UB), which consequently begins to extend, invade the adjacent metanephric mesenchyme, and branch in a stereotypic pattern (for review, see Ekblom 1996; Saxen 1999). At the same time, a group of specified progenitors within metanephric mesenchyme condenses and forms a cap around the tips of the extending and branching UB. Mutual inductive interactions between UB and metanephric mesenchyme are crucial for proper kidney morphogenesis. A population of cells within this condensed tissue further undergoes mesenchymal-to-epithelial transition and forms renal vesicles that proceed through a series of morphogenic transformations, giving rise to the S-shaped bodies, glomeruli, and, ultimately, to most of the nephron structure. The UB derivatives contribute to the collecting duct system and future ureter. Thus, the nephron represents a minimal functional unit of the kidney (Costantini 2006; Merkel et al. 2007). Wnt signaling has been proposed to play a role in kidney morphogenesis due to the expression of several Wnt ligands at the various stages of development in the Wolffian duct, UB and branch tips, and metanephric mesenchyme (Stark et al. 1994; Itaranta et al. 2002; Carroll et al. 2005; Iglesias et al. 2007). Moreover, Wnt9b, Wnt4, or Wnt11 gene ablation revealed a critical role of Wnt ligands in kidney induction, morphogenesis, and branching, and their interaction with other pathways (Stark et al. 1994; Majumdar et al. 2003; Carroll et al. 2005).

Conditional mutations of β -catenin in different cell populations of the developing kidney allowed an analysis of the role of canonical Wnt signaling in kidney morphogenesis (Table 1). Loss-of-function mutations of β -catenin were introduced in the population of progenitors within metanephric mesenchyme using Six2GFP cre (Park et al. 2007). β -Catenin mutant mice showed a reduced kidney size with very few mature nephron structures and reduced branching. The reduced expression of early mesenchymal induction markers such as Fgf8, Wnt4, Pax8, and Lhx1 was also observed. The investigators suggest that residual marker expression results from cells that have escaped recombination. Furthermore, gain-of-function mutations of β -catenin were introduced in the same cell population using Six2GFP-cre. Mutant mice showed larger kidney size with reduced branching and an absence of epithelial stages of tubulogenesis. The metanephric mesenchyme was grossly enlarged and condensed and showed ectopic and disorganized overexpression of Fgf8, Wnt4, Pax8, and Lhx1. This is in line with a study in which dominant-negative Tcf or stabilized β -catenin was overexpressed in metanephric mesenchyme in isolated organ culture (Park et al. 2007; Schmidt-Ott et al. 2007). These data indicate that activation of canonical Wnt signaling in the cap mesenchyme of nephron progenitors is sufficient to initiate an ectopic program for early inductive events. The lack of epithelial structures in gain-of-function mutants suggests that a down-regulation of Wnt signaling at later stages of development is needed for proper mesenchymal-to-epithelial transitions to occur. This study demonstrated an important role of canonical Wnt/ β -catenin signaling for the generation of the inductive signal within

the metanephric mesenchyme and the consequent development of epithelial structures of the kidney. The study of Park et al. (2007) using loss- and gain-of-function mutations of β -catenin also allowed exploring the mechanism of action of Wnt9b and Wnt4. It has been previously speculated that Wnt4 may act in noncanonical Wnt signaling in kidney development (Jho et al. 2002; Maretto et al. 2003; Osafune et al. 2006). Park et al. (2007) have demonstrated that β -catenin gain-of-function mutations can rescue the early stages of nephron induction in both Wnt9b and Wnt4 knockout mice, indicating that both these Wnt ligands act in canonical Wnt signaling in the context of kidney development. This is in line with other studies that indicate a role of Wnt4 in canonical Wnt signaling (Lyons et al. 2004; Carroll et al. 2005). In another study, loss- and gain-of-function mutations of β -catenin have been introduced in the Wolffian duct epithelium, using Hoxb7 cre (Table 1; Marose et al. 2008). Loss-of-function mutations revealed a range of phenotypes including extreme hypoplasia and cystic kidneys. The UB was completely absent in some cases, and in others, UB branching was severely reduced depending on a degree of β -catenin ablation. All mutant mice completely lacked metanephric mesenchyme differentiation as well. Adherens junctions were unaffected in the mutants, indicating that β -catenin functions in the UB and Wolffian duct through canonical Wnt signaling (see also Bridgewater et al. 2008). Expression of Ret and Wnt11, markers for UB branching, was lost. Instead, mutants prematurely expressed differentiation markers such as ZO1 α . In contrast, introduction of gain-of-function mutations of β -catenin using Hoxb7 cre resulted in a suppression of differentiation. Wnt/ β -catenin signaling is thus necessary to maintain cells of Wolffian duct/UB in an undifferentiated state, controlling the balance between undifferentiated and differentiated states during kidney development.

Lung development begins when the ventral foregut endoderm invaginates to form two lung primordial buds, which consequently begin to extend, invade the adjacent mesenchyme, and branch in a stereotypic tree-like pattern, forming the so-called respiratory bronchial tree (for review, see Hogan 1999). The endoderm gives rise to various epithelial cell types that line the inner surface of the developing lung and trachea, whereas the lung mesenchyme gives rise to the connective tissue of the lung as well as endothelial cell precursors, the smooth muscle around the airways, the cartilage of the trachea, the lymphatics, pulmonary vessels, and the mesothelial cells that cover the outer surface of the lung. Lungs are structurally and functionally divided into two distinct components: the proximal airways that conduct air and the distal airways that mediate gas exchange. Various Wnt pathway components including canonical and noncanonical Wnt ligands are expressed and appear to function at various stages of lung development, both in the lung epithelium and mesenchyme (Hogan 1999). Conditional mutations of β -catenin in mice have demonstrated that canonical Wnt signaling plays a role at different stages and tissues during lung development (Table 1). Condi-

Grigoryan et al.

tional loss-of-function mutations of β -catenin in epithelial cells of the embryonic mouse lung, using an inducible surfactant protein C promoter-driven cre (SP-C cre), resulted in pulmonary malformations with enlarged and elongated proximal airways and inhibited distal airways (Mucenski et al. 2003; Shu et al. 2005). The number of main stem bronchi and their correct branching were not affected in the mutant mice. However, the branching of secondary bronchi was perturbed. In addition, the expression of genes of proximal cells, such as Clara cell secretory protein (CCSP), was expanded, whereas the expression of the genes of distal airways such as SP-C, was reduced (Shu et al. 2005). Expression of *Bmp4* and *N-myc* was absent in mutant cells, and expression of *Fgf10* was reduced. This indicates that canonical Wnt signaling may act upstream of those genes in regulating the proximal–distal (P–D) patterning of lungs (Shu et al. 2005). Gain-of-function mutations of β -catenin, introduced in the lung epithelium using inducible CCSP promoter-driven cre (CCSP cre), demonstrated the opposite phenotype (Mucenski et al. 2005). They induced the ectopic production of peripheral lung cells in conducting airways. Mutant mice displayed epithelial hyperplasia with focal areas of inflammation and developed solid pulmonary tumors of epithelial origin. A new role for Wnt signaling in the lung mesenchyme has recently been suggested on the basis of β -catenin loss-of-function mutations introduced using *Dermo1-cre* (De Langhe et al. 2008). Mutant embryos showed shortened trachea and reduced abnormal branching. Loss of β -catenin expression affected progenitors of parabronchial smooth muscle cells. β -Catenin may act upstream of *Pitx2* in the regulation of expansion of parabronchial smooth muscle cells, as demonstrated by the loss of *Pitx2* expression in β -catenin mutants and phenotype similarity with *Pitx2*^{-/-} mice. Additionally, mutant mice had abnormally formed blood vessels in the lung, which were lined up with premature angioblasts (De Langhe et al. 2008). In conclusion, conditional loss-of-function mutations of β -catenin in lung mesenchyme demonstrated that canonical Wnt signaling plays a dual role, promoting expansion of parabronchial smooth muscle cells via binding to *Pitx2*, and controlling proper differentiation of the angioblasts into mature endothelial cells.

Like other epithelial appendages, mammary glands originate from surface ectoderm and develop through distinct epithelial–mesenchymal interactions (Robinson et al. 1999; Veltmaat et al. 2003; Chu et al. 2004). An important role of Wnt signaling in the mammary gland was suggested by Nusse and Varmus, who identified *Wnt1* (*int-1*) as a proto-oncogene in mouse mammary tumors (Nusse and Varmus 1982). A further role was demonstrated in *Lef1*^{-/-} mice, which lacked mammary anlagen (van Genderen et al. 1994). In addition, the overexpression of *Wnt1* or *Wnt10b* under the MMTV promoter resulted in mammary hyperplasias, increased proliferation and branching, and induced tumors (Tsukamoto et al. 1988; Lane and Leder 1997). Conditional gain-of-function mutations of β -catenin have been introduced in mammary alveolar epithelium using the MMTV-long

terminal repeat cre (MMTV-LTR cre) or the whey acidic protein cre (WAP cre) (Table 1; Miyoshi et al. 2002; Renou et al. 2003). β -Catenin gain-of-function mutations resulted in mammary hyperplasias, accompanied by a loss of alveolar structures. Alveolar cells failed to differentiate and instead adopted epidermal fate. No tumor formation was observed. In contrast, overexpression of other forms of stabilized β -catenin, such as Δ N57-, Δ N89-, and Δ N90- β -catenin under control of the K5 or MMTV-LTR promoter, resulted in the formation of mammary gland tumors (Imbert et al. 2001; Michaelson and Leder 2001). Precocious alveolar differentiation was also observed. The difference in phenotypes observed in these mouse models has been attributed to different levels of transcriptionally active β -catenin (Miyoshi and Hennighausen 2003). So far, no data have been reported on conditional β -catenin loss-of-function mutants in the mammary gland. In summary, different β -catenin mouse models have been successfully used to determine the involvement of Wnt/ β -catenin signaling in cell fate decisions and the proper development of the mammary gland. The data demonstrate that activated Wnt/ β -catenin signaling is dominant over intrinsic differentiation programs and can lead to an epidermal fate or neoplasias in various cell types.

Wnt/ β -catenin signaling in hematopoiesis

Hematopoietic stem cells (HSCs) are a class of multipotent stem cells that arise from common progenitors of mesodermal origin. During hematopoiesis, HSCs produce long- and short-term HSCs, which give rise to all types of differentiated blood cells of myeloid, lymphoid, and erythroid lineages (for review, see Weissman et al. 2001; Staal and Clevers 2005). Ontogenetically, hematopoiesis occurs in two major waves: primitive and definitive hematopoiesis. The first wave of primitive hematopoiesis takes place in the yolk sacs and generates transient populations of progenitors able to differentiate into nucleated erythroid and macrophage lineages. The second wave of definitive hematopoiesis gives rise to a full range of blood cells during late development and through adulthood. This population of definitive multipotent progenitors resides mainly in the fetal liver—the major hematopoietic organ in embryogenesis. In adults, the major organ for the hematopoiesis and HSC maintenance is the bone marrow (for review, see Kondo et al. 2001; Weissman et al. 2001). Canonical Wnt signaling was previously suggested to play an important role in proliferation and maintenance of HSCs, as well as in maturation of progenitors of B- and T-lymphocytes (van Genderen et al. 1994; Verbeek et al. 1995). Further evidence for the role of Wnt signaling in HSCs proliferation and self-renewal came from studies by the groups of Nusse and Weissman, who activated Wnt signaling in HSCs by stimulation with *Wnt3a* or overexpression of stabilized β -catenin (Reya et al. 2003; Willert et al. 2003). Evidence that Wnt signaling plays a role in T- and B-cell development came from studies of *Tcf1*^{-/-} and *Lef1*^{-/-} mice (van Genderen et al. 1994; Verbeek et al. 1995).

To study the role of Wnt signaling in T-cell development, β -catenin loss- and gain-of-function mutations were introduced in premature thymocytes using Lck cre (Table 1; Gounari et al. 2001; Mulroy et al. 2003; Xu et al. 2003). T-cell development was blocked in β -catenin loss-of-function mutants. In β -catenin gain-of-function mutant mice, T-cell development was accelerated; however, the mutant T cells lacked the T-cell receptor complex TCR-CD3, indicating that correct thymocyte maturation was prevented. To study the role of Wnt signaling at earlier stages in HSCs, gain-of-function mutations of β -catenin were introduced, using interferon-inducible Mx cre. This resulted in a disruption of balance between long-term and short-term HSCs, and a block of differentiation into all hematopoietic cell lineages (Kirstetter et al. 2006; Scheller et al. 2006). The HSCs lost their quiescent state and showed increased proliferation. The differentiation failure of the mutant HSCs may be a result of excessive cycling and exhaustion of the long-term HSC pool (Scheller et al. 2006). β -Catenin loss-of-function mutations using vav cre also lead to impaired self-renewal of the HSCs (Zhao et al. 2007). Surprisingly, combined absence of β -catenin and plakoglobin (γ -catenin) did not cause any defects in development of all immature and mature subsets of all hematopoietic lineages, or in reconstitution of long- and short-term HSC populations, as demonstrated in chimeric bone marrow transplants (Jeannet et al. 2008; Koch et al. 2008). Remarkably, canonical Wnt signaling remained active in the absence of β -catenin and plakoglobin (γ -catenin), as demonstrated in Wnt-reporter mice (Jeannet et al. 2008). Based on previous data that Lef1 and Tcf1, the downstream transcription factors of canonical Wnt signaling, are essential for proper hematopoiesis (van Genderen et al. 1994; Verbeek et al. 1995) and that the N-terminal domain of Tcf1, containing the β -catenin-binding site, was essential to rescue thymocyte development in Tcf1^{-/-} mice (Ioannidis et al. 2001), it has been suggested that a yet-unidentified factor capable for binding the N terminus of Tcf1 is likely to be responsible for Wnt activity in hematopoiesis in the absence of both β -catenin and plakoglobin (γ -catenin). More work is needed on this novel mechanism for Wnt pathway functions. Recently, a remarkable study by Reya and colleagues (Zhao et al. 2007) showed that β -catenin loss-of-function mutations in HSCs using vav cre prevented the development of Bcr-Abl-induced chronic myelogenous leukemia. These studies demonstrated that canonical Wnt signaling is required for the self-renewal of hematopoietic cancer stem cells.

Wnt/ β -catenin signaling in the limb

Canonical Wnt signaling also plays important roles at different stages of limb development. Limb buds of vertebrates form from limb primordia, determined groups of cells at specific locations along the embryo body. Limb bud outgrowth and patterning are established along three cardinal axes and require coordinated interactions between distinct signaling centers: The apical ectodermal

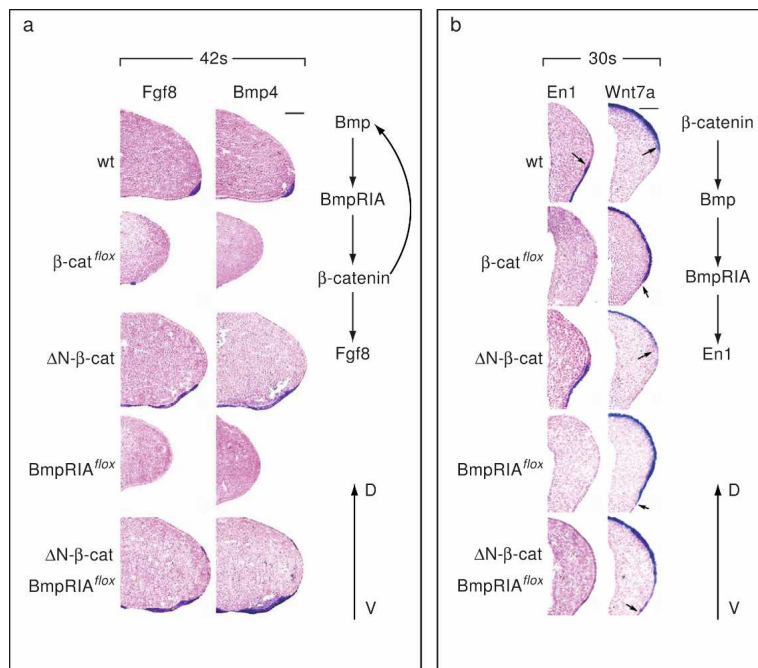
ridge (AER) controls P-D elongation of the limb buds (from shoulder to finger tip); the zone of polarizing activity (ZPA) controls A-P patterning (from thumb to little finger); and the limb bud ectoderm directs D-V axis formation (back of the hand to palm) (for review, see Capdevila and Izpisua Belmonte 2001; Niswander 2003). Wnt signals participate in the patterning of both AER and D-V axis formation. In the chick, limb formation is initiated by Wnt2b and Wnt8c in the lateral plate mesoderm to restrict the expression of Fgf10 to the prospective limb mesoderm. Fgf10 then induces the expression of Wnt3a (Wnt3 in the mouse) in the limb ectoderm that is required for Fgf8 expression in the presumptive AER (Kawakami et al. 2001; McQueeney et al. 2002; Barrow et al. 2003).

In order to study the role of Wnt/ β -catenin signaling in the surface ectoderm of limbs, loss- and gain-of-function mutations of β -catenin were produced using Msx2 cre (Barrow et al. 2003) or Brain 4 cre (Table 1; Soshnikova et al. 2003). Cre-induced loss-of-function mutation of β -catenin resulted in severe malformations or complete absence of limbs. In limbs of mildly affected mutants, ventral dermal pads were absent, and circumferential nails were detected. The mutant mice thus displayed an absence of ventral and duplication of dorsal structures. These data indicated that Wnt/ β -catenin signaling is essential in the surface ectoderm of limbs for both AER (P-D axis) and D-V axis formation. These phenotypes are similar to those obtained when the BmpR1a was ablated conditionally (Ahn et al. 2001). In order to analyze the epistatic interaction between Wnt and Bmp signaling in P-D and D-V patterning of the limb, our lab generated compound mutants in which conditional gain-of-function mutations of β -catenin and loss-of-function mutations of the BmpR1a were introduced in the limb ectoderm, using Brain 4 cre (Soshnikova et al. 2003). Remarkably, in these compound mutants, the area of the AER, the expression domains of Fgf8 and Bmp4, and the overall size of the limbs were strongly enlarged (Fig. 8a). This was also observed in embryos that carry only the β -catenin gain-of-function mutation. These data demonstrated that Wnt/ β -catenin signaling is located downstream from Bmp signaling in AER (P-D) patterning of the limb. Moreover, we examined these compound mutant embryos for the expression of Engrailed 1 (En1) and Wnt7a, which are expressed along the D-V axis. Remarkably, in the compound mutants, En1 expression in the ventral ectoderm was absent, and Wnt7a was expressed in both dorsal and ventral ectoderm, as it was observed in single loss-of-function mutants (Fig. 8b). Thus, both ectodermal β -catenin and BmpR1a are required for correct specification of the ventral character in limb ectoderm. However, enhanced β -catenin signaling does not rescue the deficits caused by the BmpR1a loss-of-function mutation, suggesting that genetically, β -catenin acts upstream to the BmpR1a during D-V patterning (Soshnikova et al. 2003).

The finding that the hierarchies of Wnt and Bmp signaling were different in the formation of two different axes in the limb was remarkable. It showed that in the

Grigoryan et al.

Figure 8. Wnt/ β -catenin signaling in limb patterning. (a) At 42 somite stage AER is absent or severely reduced in β -catenin loss-of-function mutants (β -cat^{fllox}), and expanded in the gain-of-function mutants (Δ N- β -cat), as shown by Fgf8 and Bmp4 expression. AER is not formed in BmpR1a loss-of-function mutants (BmpR1A^{fllox}) at 42 somite stage. In compound mutants with loss-of-function mutations of BmpR1a and gain-of-function mutations of β -catenin AER is expanded, resembling the phenotype of single β -catenin gain-of-function mutants. This indicates that canonical Wnt signaling acts downstream from Bmp signaling in AER formation. (b) At 30 somite stage expression of the ventral gene En1 is lost and expression of the dorsal gene Wnt7a is expanded ventrally in β -catenin loss-of-function mutants. In contrast, in gain-of-function mutants, expression of these markers is unchanged. In BmpR1a loss-of-function mutants, as well as in compound mutants, phenotypes resemble that of β -catenin loss-of-function mutants, indicating that canonical Wnt signaling acts upstream of, or in parallel to the Bmp signaling in D-V patterning of the limb. All gene mutations were introduced using Brain 4 cre. Reprinted with permission from Soshnikova et al. (2003).



development of one organ, hierarchies of signaling pathways can be different in particular processes, even if they occur at the same time. The intricate interactions between Wnt/ β -catenin and Bmp signaling in the limb ectoderm provide the molecular basis that ensures a tight spatial-temporal control of signaling responses and may connect the development of P-D and D-V axes.

Conclusions: lessons from conditional loss- and gain-of-function β -catenin mutant mice

Genetic analysis in mice, particularly the use of conditional loss- and gain-of-function mutations of β -catenin in various tissues and organs, demonstrated that canonical Wnt signals play key roles in the maintenance and expansion of embryonic progenitor cells and stem cells. Of particular interest are recent findings on the functions of canonical Wnt signals in the maintenance of cancer stem cells. For instance, in ras-induced squamous cell carcinomas of the skin, loss-of-function mutations of β -catenin lead to tumor regression, demonstrating that maintenance of the cancer stem cell population is dependent on β -catenin signaling. Conversely, an increase in the number of mammary cancer stem cells was found in premalignant mammary glands of MMTV-Wnt1 transgenic mice (Shackleton et al. 2006). These functions of Wnt signaling in cancer stem cells of the skin and mammary gland are analogous to those described recently in cancer stem cells of the hematopoietic system.

In addition, Wnt/ β -catenin signals help direct cells toward particular fates. This review includes examples of loss- and gain-of-function mutations, where canonical Wnt signals instruct cells to generate hair but not epi-

dermis (skin), enterocytes and Paneth cells but not goblet cells (intestine), osteoblasts but not chondrocytes (bone), and the second but not the first heart field. In several tissues, like the intestine, the pituitary, and the nervous system, Wnt signals control in parallel self-renewal of those progenitors, which they also direct to adopt a particular fate. In particular, in the nervous system, Wnt signals direct differentiation, for instance, by directing a sensory neuronal fate or pattern in the spinal cord. In parallel, they allow the maintenance and expansion of the corresponding progenitor pool. Similarly, in the pituitary, Wnt signaling maintains the progenitor pool and directs differentiation of the Pit1-expressing lineages. The opposing effects of the β -catenin gain- and loss-of-function mutations are particularly useful in the characterization of such dual functions: Frequently, the instructive role of canonical Wnt signaling can be analyzed only by gain-of-function mutations, which show the expansion of lineages at the expense of others, while in loss-of-function mutations, the depletion of a progenitor pool prevents such an analysis.

Studies of these themes have been complicated by the dual role β -catenin can have in canonical Wnt signaling and in cadherin-mediated cell adhesion (Takeichi 1991; Kemler 1993; Gates and Peifer 2005). In many cases, β -catenin loss-of-function mutations affect the integrity of the targeted tissues. It had been shown that plakoglobin (γ -catenin), which is the closest relative of β -catenin in the Armadillo protein family, can take over the function of β -catenin in cell adhesion in the loss-of-function β -catenin mutations. This happens, for instance, in the early mouse embryo (Huelsenken et al. 2000), in the epidermis (Posthaus et al. 2002), or in the heart (Zhou et al. 2007). In other cases, mutations of β -catenin clearly re-

sulted in disintegration of tissues due to defective adhesion (Junghans et al. 2005; Smith et al. 2005). In each single case, it must be determined whether the effects of β -catenin are mediated by Wnt-controlled transcriptional events or by cell adhesion defects. Here gain-of-function mutations of β -catenin have been essential in revealing the role of canonical Wnt signaling, by producing phenotypes opposite to loss-of-function experiments.

A range of target genes of Wnt/ β -catenin signaling have been identified (see also <http://www.stanford.edu/~rnusse/wntwindow.html>), but so far only a few have been proven to be direct and/or essential targets. For instance, the negative regulator of canonical Wnt signals, Axin2/Conductin, is a direct target. Our Axin2–LacZ reporter mouse, which expresses lacZ under the control of the endogenous Axin2 promoter, has turned out to be very useful to monitor endogenous canonical Wnt signals in many tissues and organs (Lustig et al. 2002; Soshnikova et al. 2003; Zechner et al. 2003, 2007). It has been a decade since c-Myc was identified as a direct target of canonical Wnt signals in the intestine. Recently, a thorough mechanism of regulation of the c-Myc promoter by the β -catenin transcriptional complex has been described (Sierra et al. 2006). The physiological importance of c-Myc as an essential Wnt target was also corroborated genetically: The conditional ablation of c-Myc rescued the phenotype of the APC deletion in the intestine. Another gene, Pitx2, was identified as a direct and essential target of canonical Wnt signaling in the pituitary gland, the heart, the pancreas, and the lung. Further essential targets of Wnt signaling include Mitf1 in the melanocyte lineage, Olig3 in the dorsal spinal cord, Ptf1a in the pancreas, and Fgf10 in the second heart field, the pancreas, and lung. Finally, we should mention two exotic cases: Prop1 is a transcription factor that directly interacts with β -catenin instead of Tcf/Lef factors to control Wnt/ β -catenin-dependent signaling in particular pituitary sublineages. And in hematopoiesis, Wnt/Tcf-dependent target gene activation can occur independently of β -catenin and plakoglobin (γ -catenin). Comprehensive identification of direct and essential targets of canonical Wnt signaling, and of the mechanisms by which β -catenin regulates the transcription of those genes, remains an important aspect to address in the future.

We discussed several examples in which Wnt/ β -catenin and other signaling systems act on each other in a hierarchical manner. The hierarchies in which these signaling pathways interact can vary. For instance, in the establishment of the axes in the limb, Bmp signals act upstream of or downstream from Wnt/ β -catenin, depending on the context. In addition, the multipotent state of neural crest cells requires activity of both Wnt and Bmp signaling, and Bmp appears to antagonize the signal provided by Wnt/ β -catenin. Wnt and Bmp signals also cooperate to maintain quiescent skin stem cells. Oscillating expression of Bmps in normal skin regulates oscillations in Wnt signaling to ensure periodic activation of the bulge stem cells and allow coordinated hair differentiation during the hair cycle. Oscillations of Wnt and Notch signaling activities in strictly opposite phases

have been demonstrated to be crucial for somitogenesis. This indicates that the mechanism must be tightly regulated. During skeletogenesis, commitment within the osteoblast lineage requires strictly regulated stage-specific genetic interaction between Wnt and Indian Hedgehog (Ihh) pathways. Apparently in osteoblast differentiation, β -catenin acts downstream from Ihh signaling, whereas in chondrocyte survival, β -catenin acts upstream of Ihh to inhibit chondrocyte apoptosis. Sonic hedgehog acts upstream of Wnt/ β -catenin in the taste bud specification. These intricate interactions of canonical Wnt with other signaling systems provide the molecular basis for tight spatio-temporal control of the consecutive steps of developmental processes.

In cases in which specific cre mice have been available, studies of β -catenin loss of function and gain of function have been fruitful. In the future, these studies can be extended to studying the role of canonical Wnt signaling in further aspects of embryonic development, for example, in the brain, thanks to the ongoing development of many more cre mice (for instance, see <http://www.gensat.org/cre.jsp>). This will allow us to study the pathway's effects on specific precursor populations and will permit a precise mapping of the regulatory networks of developmental signaling pathways in the different tissues and organs and at particular time points, as well as in regulating adult stem cells in organs such as the skin, intestine, and the hematopoietic system. Regulatory networks are also complex in tumors: For instance, Wnt/ β -catenin signaling acts downstream from ras signaling in epidermal tumors (Malanchi et al. 2008), which is opposite to the situation in intestinal tumors (Kinzler and Vogelstein 1996). Deeper research into canonical Wnt signaling will be needed to deal with these complexities, as well as to reveal further direct and essential target genes of the Wnt pathway. We expect that the large collection of mouse models presently available, in particular, the gain-of-function mutants, will be of use for the testing and improvement of Wnt-modulating agents.

Finally, it is important to remember that conditional mutagenesis of β -catenin was first used in 1999, but important functions of canonical Wnt signals in several tissues were identified earlier. For instance, in the intestine, a great deal of information on the role of Wnt signaling was generated in the 1990s by the analysis of APC functions in tumor formation. However, very important recent findings obtained from the use of the loss- and gain-of-function mutations in β -catenin reveal the role of canonical Wnt signaling in stem cell and cancer stem cell maintenance and lineage decisions. Altogether, the use of conditional β -catenin mutant mice has demonstrated that canonical Wnt signals are used over and over again in development and maintenance of organs. It remains astonishing to us that such a wide array of diverse biological functions is mediated by a single signaling mechanism. On the other hand, the system provides an excellent model of the way evolution has used a module over and over again to achieve a wide variety of similar—yet subtly unique—effects within the tissues of multicellular organisms. As our knowledge of the uses and

Grigoryan et al.

regulation of this system deepens, this will permit the development of further powerful animal models of human development and disease processes.

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Grigoryan et al.

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