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DEVELOPMENT AT A GLANCE

Wnt signaling in development and tissue homeostasis

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ABSTRACT

The Wnt-β-catenin signaling pathway is an evolutionarily conserved cell-cell communication system that is important for stem cell renewal, cell proliferation and cell differentiation both during embryogenesis and during adult tissue homeostasis. Genetic or epigenetic events leading to hypo- or hyper-activation of the Wnt-β-catenin signaling cascade have also been associated with human diseases such as cancer. Understanding how this pathway functions is thus integral for developing therapies to treat diseases or for regenerative medicine approaches. Here, and in the accompanying poster, we provide an overview of Wnt-β-catenin signaling and briefly highlight its key functions during development and adult tissue homeostasis.

KEY WORDS: Wnt protein, Wnt signaling, Epigenetics

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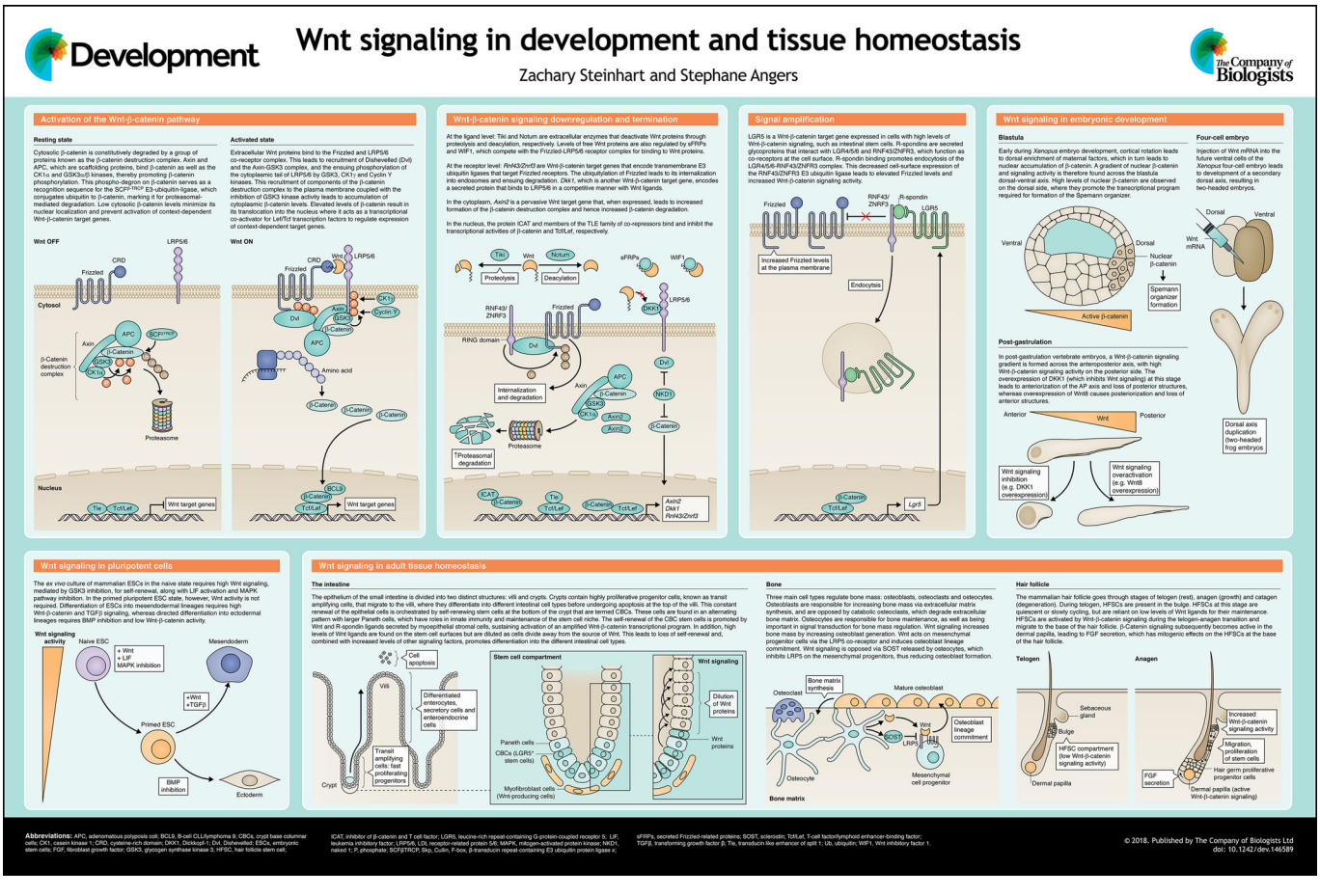
Introduction

Wnt proteins are secreted growth factors that regulate the proliferation and differentiation of stem and progenitor cells, both during embryonic development and during adult tissue homeostasis in multicellular animals (Logan and Nusse, 2004). The *Drosophila* Wnt protein Wingless (Wg) and the core members of the intracellular signaling pathway it regulates were originally identified and characterized in flies through genetic screens and phenotypic analyses of embryonic morphogenesis defects (Nüsslein-Volhard and Wieschaus, 1980; `Sharma and Chopra, 1976). The discovery that Wg was homologous to the mouse *Wnt1* proto-oncogene (Rijsewijk et al., 1987) supported the notion that Wnt1 and possibly other vertebrate Wnt homologs could have important roles during normal vertebrate development, and that Wnt signaling activity might be dysregulated in cancer. A vast number of integrative genetic, biochemical and 'omic-based approaches have since refined our understanding of how Wnt ligands are produced and secreted, how cells recognize and integrate Wnt signals to yield various cellular and physiological responses, and how Wnt signals are modulated, terminated or



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amplified depending on spatiotemporal contexts. This work has provided a foundation to recognize and study the pervasive roles of Wnt signaling during development and tissue homeostasis, as well as to understand the frequent defects associated with this pathway in human diseases such as cancer (Nusse and Clevers, 2017).

Multiple functionally divergent Wnt-based signaling pathways have been identified to date. The best characterized of these is the Wnt-β-catenin pathway, which is often referred to as the 'canonical' Wnt pathway. This pathway culminates in the regulation of context-specific β-catenin-dependent gene expression programs that direct stem and progenitor cell renewal, proliferation and differentiation. By contrast, the non-canonical pathways operate independently of β-catenin and have been described to impinge on small GTPases of the Rho family to control cell polarity and cell movement, or to act via heterotrimeric G proteins to control Ca^{2+} signaling (Angers and Moon, 2009). Here, and in the accompanying poster, we discuss the key features of the canonical Wnt-β-catenin pathway and briefly highlight its roles in development, stem cells and adult tissue homeostasis.

Wnt signal recognition and pathway initiation

The Wnt-β-catenin signaling pathway centers around the post-translational control of β-catenin protein abundance. In the absence of Wnt proteins, cytoplasmic levels of β-catenin are kept low through ubiquitin-dependent proteasomal degradation, a process governed by a molecular machine called the β-catenin destruction complex (Stamos and Weis, 2012). The destruction complex is composed of the scaffolding proteins Axin (Behrens, 1998; Hart et al., 1998) and APC (Munemitsu et al., 1995; Rubinfeld et al., 1993), and the kinases CK1α (Amit et al., 2002; Liu et al., 2002; Rubinfeld et al., 1996) and GSK3α/β (Amit et al., 2002; Liu et al., 2002; Rubinfeld et al., 1996). The destruction complex functions by catalyzing the serine/threonine phosphorylation of a highly conserved phospho-degron at the N terminus of β-catenin (Winston et al., 1999), which earmarks β-catenin for recruitment to the $SCF^{\beta\text{-}TRCP}$ E3-ubiquitin ligase (Hart et al., 1999) and ensuing proteasome-mediated degradation (Hart et al., 1999). Simply put, in the absence of Wnt proteins, neo-synthesized β-catenin is constitutively targeted for proteolysis, and Wnts function by inhibiting this degradation.

Wnts are proteins of ∼40 kDa that are post-translationally modified through palmitoylation and glycosylation. The human genome encodes 19 Wnt proteins (Nusse, 2001), all of which are secreted through a conserved secretory pathway. Wnt proteins are translated in the endoplasmic reticulum (ER), where they are palmitoylated by the ER-bound o-acyl-transferase porcupine (van den Heuvel et al., 1993; Willert et al., 2003). This is an essential step in Wnt protein secretion (Herr and Basler, 2012) and introduces a modification that is required for Wnt activity (Janda et al., 2012; Proffitt and Virshup, 2012; Willert et al., 2003). Following their palmitoylation Wnt protein interact with the multipass transmembrane escort protein GPR177, the vertebrate homolog of *Drosophila* Wingless/Evi/Sprinter, which mediates endosome trafficking to the plasma membrane and the release of Wnt proteins into the extracellular space (Bänziger et al., 2006; Bartscherer et al., 2006; Goodman et al., 2006).

The cell-surface receptor for Wnts that is responsible for transducing signals through the β-catenin pathway consists of a heterodimer between a Frizzled family member (Bhanot et al., 1996; MacDonald and He, 2012; Yang-Snyder et al., 1996) and one of the Wnt co-receptors LRP5 or LRP6 (LRP5/6) (Pinson et al., 2000; Tamai et al., 2000; Wehrli et al., 2000). Frizzled proteins are seven-transmembrane receptors, of which there are 10 homologs in humans, which all contain a conserved 120 amino acid extracellular cysteine-rich-domain (CRD) serving as the obligate binding interface for Wnt (Janda et al., 2012; Povelones and Nusse, 2005). Structural studies have revealed that Wnt proteins resemble the general structure of a hand, with the thumb and index fingers pinching the extracellular CRD of Frizzled. The Wnt palmitoleic acid moiety acts as a major binding determinant, inserting itself in a hydrophobic crevice within the CRD (Janda et al., 2012). LRP5/6 Wnt co-receptors, which are single transmembrane domain proteins, contain four extracellular β-propeller domains that are involved in Wnt binding (Chen et al., 2011) and five PPPSP phosphorylation motifs within the intracellular region (Tamai et al., 2004).

The binding of a Wnt ligand to the FZD-LRP5/6 complex first results in recruitment of the cytoplasmic protein Dishevelled (Klingensmith et al., 1994; Krasnow et al., 1995), putatively via direct interaction with Frizzled (Cong et al., 2004; Tauriello et al., 2012), and of Axin-GSK3 complexes via the interaction of Axin with Dishevelled. GSK3 (Tamai et al., 2004) and the Cdk14(PFTK1)-Cyclin Y mitotic kinase complex (Davidson et al., 2009) then phosphorylate PPPSP motifs within the intracellular region of LRP5/6, thereby priming them for further phosphorylation by the membrane-anchored kinase casein kinase 1 γ (Davidson et al., 2005; Zeng et al., 2005). The phosphorylated C-terminal tail of LRP5/6 was thought to represent a high-affinity binding site for Axin, thereby leading to the titration of active destruction complex molecules and to β-catenin stabilization. However, this model mostly relied on yeast two-hybrid experiments that identified Axin clones using the LRP5 tail as a bait (Mao et al., 2001); subsequent biochemical (Cselenyi et al., 2008; Piao et al., 2008) and structural experiments (Stamos et al., 2014) have demonstrated that phospho-LRP5/6 blocks β-catenin degradation directly by inhibiting GSK3 activity. Whether Axin interacts with the phosphorylated LRP5/6 intracellular tail indirectly through GSK3, or whether direct binding of Axin is needed, remains to be determined.

The subsequent accumulation of β-catenin leads to its translocation to the nucleus, where it acts as a transcriptional co-activator by binding to and modulating the activity of the Tcf/Lef family of DNA-binding proteins (Behrens et al., 1996; Molenaar et al., 1996; van de Wetering et al., 1997) that otherwise repress Wnt-responsive enhancers through their physical interaction with Groucho/Tle family of co-repressors (Cavallo et al., 1998; Roose et al., 1998). How this transcriptional switch functions at the molecular level was recently clarified with the identification of the E3 ubiquitin ligase UBR5 as a required component of the Wnt-dependent response (Flack et al., 2017). UBR5 functions through the Wnt- and ubiquitin-mediated inactivation of Groucho/Tle, thereby leading to de-repression and activation of β-catenin target genes in a context-dependent manner. Wnt-β-catenin target genes vary depending on cell lineage/type (Nakamura et al., 2016), but common targets include genes that function in positive- and negative-feedback regulation of the pathway, genes involved in cell-cycle progression and genes involved in stem cell function.

Wnt signal restriction and termination

Multiple mechanisms exist to restrict and/or terminate Wnt signaling at the ligand, receptor, intracellular and nuclear levels. At the ligand level, the extracellular enzymes Tiki and Notum function as a protease and a carboxylesterase to cleave the N terminus of Wnts (Zhang et al., 2012) or remove their palmitoleate

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moiety (Kakugawa et al., 2015; Zhang et al., 2015), respectively. Additionally, Wnt ligands are inhibited through direct interaction with secreted proteins that prevent their interaction with the receptor complex, such as Wnt inhibitory factor (WIF) (Hsieh et al., 1999), or secreted frizzled related proteins (sFRPs) (Hsieh et al., 1999; Leyns et al., 1997; Rattner et al., 1997; Wang et al., 1997), which share homology with the Frizzled CRD.

Several negative-feedback loops exist to terminate or dampen Wnt signals at the receptor level. Dickkopf-1 (*Dkk1*) (Glinka et al., 1998) is a Wnt-β-catenin target gene (González-Sancho et al., 2005) and functions by binding to LRP5/6 in competition with Wnts (Bafico et al., 2001; Semënov et al., 2001). *Rnf43* and *Znrf3* are recently characterized Wnt-β-catenin target genes that encode transmembrane E3-ubiquitin-ligases (Hao et al., 2012; Koo et al., 2012) involved in ubiquitin-dependent Frizzled endocytosis and proteasomal degradation, thereby reducing receptor levels at the plasma membrane.

In the cytoplasm, the best-studied negative feedback for β-catenin levels involves Axin2. Both Axin1 and Axin2 are functionally equivalent proteins (Chia and Costantini, 2005) but, in contrast to its paralog *Axin1*, which is expressed constitutively, *Axin2* is a Wnt target gene (Jho et al., 2002; Lustig et al., 2002). Given that Axin proteins are thought to be limiting components of the β-catenin destruction complex (Lee et al., 2003), the rise in Axin levels in response to Wnt signaling leads to increased destruction complex formation and β-catenin degradation, thereby providing a mechanism for negative feedback (Goentoro and Kirschner, 2009). The Wnt target gene *Nkd1* also provides negative feedback in the cytoplasm but the exact mechanism by which it functions remains unclear. NKD1 was first suspected to bind to and inhibit Dishevelled (Rousset et al., 2001); however, other findings have shown that it interacts directly with β-catenin and prevents its nuclear import (Van Raay et al., 2011). Finally, the 81 amino acid protein inhibitor of β-catenin and T cell factor (ICAT) was found to directly interact with and inhibit β-catenin in the nucleus, acting to inhibit the posteriorizing activity of Wnt-β-catenin signaling in a spatiotemporal manner in the nervous system (Satoh et al., 2004).

Wnt signal amplification

A number of recent studies have revealed a mechanism involving the cell surface protein LGR5 and secreted R-spondin proteins that provides a way of augmenting signaling through the Wnt pathway. *Lgr5*, which is among the best-characterized target genes of the Wnt-β-catenin signaling pathway, is an established stem cell marker (Barker et al., 2007). As such, *Lgr5* reporter mice have proven valuable for the identification, isolation and functional characterization of tissue stem cells (Barker et al., 2007). However, the precise role of LGR5 and its homologs LGR4/LGR6 remained controversial until the discovery that they function as receptors for four R-spondin secreted proteins, RSPO1-RSPO4 (Carmon et al., 2011; de Lau et al., 2011; Glinka et al., 2011). It was further revealed that R-spondins do not possess signaling activity on their own (Janda et al., 2017) but rather amplify Wnt signaling activity, as they require the presence of Wnt ligands. A missing link in our understanding of RSPO-LGR4/5/6 function came with the identification that the E3 ubiquitin ligases RNF43/ZNRF3 act as co-receptors for RSPOs (Chen et al., 2013; Hao et al., 2012; Zebisch et al., 2013). As mentioned above, *Rnf43* and *Znrf3* are themselves target genes of Wnt-β-catenin signaling, and when upregulated are part of a negative-feedback loop that downregulates cell-surface levels of Frizzled-LRP5/6 complexes. Thus, the binding of R-spondin to the LGR4/5/6-RNF43/ZNRF3 complex leads to endocytosis of the complex and therefore blocks the ability of RNF43 and ZNRF3 to downregulate Frizzled at the cell surface, hence leading to a sensitized Wnt signaling state (Hao et al., 2012). Interestingly, R-spondin may also have roles that are independent of LGR proteins as RSPO2 and RSPO3 were found to have remaining activities in LGR4/5/6 triple-knockout cells (Lebensohn and Rohatgi, 2018). In this case, R-spondin activity is instead mediated by ZNRF3/RNF43 and heparan sulfate proteoglycans.

Interestingly, a recent study revealed that Wnt and R-spondin have non-overlapping roles during stem cell homeostasis (Yan et al., 2017). This study indicated that, whereas R-spondin overexpression is sufficient to expand the $Lgr5^{+}$ stem cell population in the intestine, increasing Wnt ligand levels is ineffective. This highlights a unique role for R-spondin in providing the high levels of Wnt signaling activity required to drive stem cell proliferation and self-renewal. These results led the authors to propose a model in which Wnt ligands act as priming factors, enabling expression of the R-spondin receptors LGR5 and RNF43/ZNRF3. Within this model, primed cells exposed to R-spondin would be competent to produce the high Wnt-β-catenin signaling response and activate transcriptional programs required for cell proliferation and self-renewal. On the other hand, cells further away from the source of R-spondin (the stem cell niche) have higher levels of functional RNF43/ZNRF3 at their surface and, as a result, gradually express decreasing amounts of Frizzled receptors, which is translated into decreased levels of Wnt-β-catenin signaling.

Wnt signaling in early vertebrate embryonic development

Cell-cell communication is integral during the embryonic development of multicellular organisms, acting to coordinate stem cell self-renewal, cell fate decisions, cell migration and the organization of cells into tissues. The Wnt-β-catenin signaling pathway is one of the evolutionary conserved communication systems that regulates embryonic development. It involves Wnt ligands that are released by Wnt-producing cells and that act over various ranges to influence neighboring Wnt-responsive cells.

Wnt-β-catenin signaling is instrumental for defining the dorsoventral and anteroposterior body axes in multiple animal species (Genikhovich and Technau, 2017; Niehrs, 2010). For example, following *Xenopus* egg fertilization, cortical rotation leads to dorsal translocation of maternal determinants that are important for β-catenin signaling activation, which in turn leads to specification of the Spemann organizer (Moon and Kimelman, 1998) – an essential embryonic inducer of dorsoventral patterning. Accordingly, the injection of Wnt mRNA or other factors that activate β-catenin signaling into future ventral blastomeres notoriously leads to duplication of the dorsal axis and results in two-headed frog embryos (McMahon and Moon, 1989). Whether Wnt ligands themselves were involved in dorsal axis induction in *Xenopus* remained controversial for a long time, but Wnt-11 was eventually identified as the ligand implicated in this context (Tao et al., 2005). At the onset of gastrulation, Wnt ligand-dependent β-catenin signaling then activates a largely distinct transcriptional program that directs anteroposterior axis development. In this context, Wnt-β-catenin signaling is inhibited anteriorly, leading to the development of head structures, and activated posteriorly to define tail formation (Green et al., 2015). Paramount to these patterning events, the spatiotemporal regulation of Wnt-β-catenin transcriptional programs is achieved through the action of secreted antagonists, epigenetic regulation, the actions of different Tcf/Lef factors and signaling integration with other

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developmental pathways. Beyond these early roles in establishing the embryonic body axes, Wnt-β-catenin signaling plays important roles during the morphogenesis of multiple tissues derived from the three germ layers.

Wnt signaling in embryonic stem cells

The derivation of human embryonic stems cells (ESCs) and induced pluripotent stem cells (iPSCs) has provided a system for studying human embryonic development and human diseases, and a framework for the production of large quantities of differentiated cells that can be transplanted for regenerative medicine or tissue engineering applications. The directed differentiation of such pluripotent stem cells (PSCs) into specialized cells in culture follows the same principles underlying embryonic development; as such, insights gained from developmental biology have guided strategies for using growth factors and inhibitors of developmental signaling pathways to maintain pluripotency or steer cell fate towards the desired lineage with increasing efficiencies (Williams et al., 2012).

Functional redundancy between Wnt-β-catenin signaling activation, leukemia inhibitory factor (LIF) signaling activation and MAPK pathway inhibition is important for maintaining the naive pluripotent state in ESCs. Indeed, β-catenin KO ESCs maintain their pluripotency when cultured with LIF but rapidly exit the naive state in its absence (Lyashenko et al., 2011). Reciprocally, the use of GSK3 inhibitors to stimulate β-catenin signaling bypasses the requirement for LIF in this process (Sato et al., 2004). Wnt signaling is therefore not absolutely required for the maintenance of pluripotency, a finding supported by the analysis of porcupine KO mESCs that exhibit normal self-renewal properties (Biechele et al., 2013). Paradoxical reports implicating Wnt-β-catenin signaling in the self-renewal versus differentiation of ESCs have been reconciled with its seemingly opposite roles in promoting pluripotency in the naive state (Sato et al., 2004; ten Berge et al., 2011; Xu et al., 2016) and differentiation in the primed state (Davidson et al., 2012; Kurek et al., 2015), which is characterized by a distinct epigenetic and gene expression landscape (Nichols and Smith, 2009). Levels of Wnt-β-catenin signaling therefore appear to control the transition between the naive and primed ESC states. In line with its role in embryonic development, the further differentiation of ESCs into different germ layers requires the temporal activation of Wnt-β-catenin signaling to steer cells towards the mesendoderm lineage or inactivation to obtain neuroectoderm (Tabar and Studer, 2014).

Wnt signaling in adult tissue homeostasis

The self-renewal versus differentiation of stem cells is directed by extrinsic short-ranged signals that emanate from the niche. Confirming the pervasive role of Wnt ligands as niche factors, lineage-tracing experiments using Wnt-target gene reporters have revealed labeled stem cells in several tissues, including the intestine (Barker et al., 2007), the stomach (Barker et al., 2010), the skin (Lim et al., 2013), the liver (Wang et al., 2015) and the mammary gland (van Amerongen et al., 2012). Below, we describe three of the best-studied tissues in which Wnt signaling has been demonstrated to play crucial roles with regard to the function of tissue stem cells.

Intestine

The best-characterized function of Wnt signaling in adult tissues is in the maintenance of stem cell niches, where Wnt ligands promote the proliferation and self-renewal capability of tissue-specific stem cells (Clevers et al., 2014). This has been particularly well studied in the adult intestinal epithelium, which turns over fully every 4-5 days and thereby requires balanced stem and progenitor cell self-renewal, proliferation and differentiation (Clevers, 2013). The intestinal epithelium is composed of two compartments: the villi, which are responsible for the absorptive and secretion functions of the intestine; and the crypts of Lieberkühn, which are not exposed to the intestinal lumen, and contain tissue stem cells and rapidly proliferating transit amplifying cells. Genetic studies have shown that the disruption of *Tcf4* (Korinek et al., 1998) or β-catenin (Fevr et al., 2007), or the overexpression of *Dkk1* (Fevr et al., 2007; Pinto et al., 2003), leads to robust loss of transit amplifying cells and crypt structures, confirming the functional requirement of Wnt-β-catenin signaling in the intestinal epithelium. These genetic experiments are corroborated by the fact that *APC* loss-of-function mutations induce adenoma formation (Morin, 1997) and occur in 80% of colorectal cancers (Cancer Genome Atlas Network, 2012). Studies on the expression of Wnt target genes have identified a Wnt signaling gradient, with highest expression in the base of the crypt, particularly in the crypt base columnar cells (CBCs) (Kosinski et al., 2007; Van der Flier et al., 2007). Wnts are bound to the plasma membrane of cells at the base of the crypt and are diluted with each cell division away from the base, thereby establishing a gradient (Farin et al., 2016). Underlying the CBCs at the bottom of crypts are myoepithelial cells, which have been shown to be the *in vivo* source of Wnt ligands (Kabiri et al., 2014). In addition, the Wnt target gene *Lgr5* was found to be expressed exclusively in CBCs meaning that it could be used for lineage-tracing experiments; these experiments showed that $Lgr5^+$ CBCs could differentiate into all epithelial cell types (Barker et al., 2007). Further supporting the possibility that $Lgr5^+$ CBCs are the intestinal tissue stem cells, dissociated single $Lgr5^+$ cells were found to give rise to 'mini-gut' organoids *in vitro*, which contain all intestinal cell types (Sato et al., 2009).

Bone

Bone tissue remodeling is crucial for maintaining a balance between systemic calcium homeostasis and the biomechanical needs of the skeleton. Human genetics data perhaps best highlight the crucial role of Wnt-β-catenin signaling in bone tissue homeostasis. Indeed, rare pathological mutations within genes encoding components of the Wnt-β-catenin pathway lead to either severely increased or decreased bone mass. For example, *Lrp5*, which encodes a Wnt co-receptor, was revealed as a critical gene directing bone mass regulation when *LRP5* loss-of-function mutations were identified in the low bone mass disorder osteoporosis-pseudoglioma syndrome (Gong et al., 2001) and when heterozygous missense mutations in *LRP5* were observed in individuals with dominantly inherited high bone mass (Boyden et al., 2002; Little et al., 2002). Another example is mutations discovered in *SOST* (sclerostin), a negative regulator of LRP5/6 that was found to be causally linked to high bone density pathology via loss-of-function mutations (Brunkow et al., 2001) or mutations that decrease its expression (Balemans et al., 2002; Loots et al., 2005). Corroborating this human genetic evidence are extensive genetic experiments performed in mice that link Wnt-β-catenin signaling to the regulation of bone homeostasis (Baron and Kneissel, 2013). Leveraging these findings, several pharmaceutical companies have developed anti-SOST antibodies to activate endogenous Wnt-β-catenin circuitries in bone tissues, and these have been shown in clinical trials to augment bone mass density and prevent fractures (Glorieux et al., 2017; Recker et al., 2015; Saag et al., 2017; Williams, 2017). At the cellular level, the process of bone remodeling results from the concerted actions of three different cell types: osteoblasts, which are responsible for

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bone matrix formation; osteoclasts, which are responsible for bone resorption; and osteocytes, which are responsible for bone maintenance. The precise roles of Wnt-β-catenin signaling in bone mass homeostasis still remain to be clarified but the prevailing model is that it functions to regulate osteoblast differentiation and function (Bennett et al., 2005; Cui et al., 2011; Day et al., 2005; Hill et al., 2005; Tu et al., 2015).

Skin

The best-studied role of Wnt signaling in adult skin is its role in the hair follicle cycle, where Wnt is an important factor in orchestrating the control of hair follicle stem cells (HFSCs) (Veltri et al., 2018). The adult hair follicle goes through continuous cycles of telogen (rest), anagen (growth) and catagen (degeneration). The essential role of Wnt-β-catenin in hair follicle cycling was identified through the observations that loss of epidermal β-catenin or inhibition of LRP5/6 via ectopic DKK1 expression leads to defects in hair follicle development and/or loss after a single hair cycle (Andl et al., 2002; Huelsken et al., 2001). Specifically, Wnt-β-catenin signaling acts on multiple cell types to induce the telogen-anagen transition (Greco et al., 2009; Kishimoto et al., 2000; Van Mater et al., 2003). During telogen, HFSCs are slow cycling or quiescent, reside in the bulge region of the hair follicle (Cotsarelis et al., 1990; Lyle et al., 1998), are LGR5-positive (Jaks et al., 2008) and rely on autocrine Wnt for maintenance (Lim et al., 2016). During the telogen-anagen transition, Wnt-β-catenin activity in the bulge HFSCs increases (Lien et al., 2014) and cells from the bulge migrate to the base of the hair follicle and begin proliferating rapidly to produce cell lineages of a new hair follicle (Oshima et al., 2001; Tumbar et al., 2004). This proliferation is aided by concurrent Wnt-β-catenin activation in the dermal papilla, which induces growth-promoting FGF signals (Enshell-Seijffers et al., 2010).

Perspectives

Wnt ligands are crucial for the development and homeostasis of virtually all tissues. In the past few decades, genetic, biochemical and structural experiments have identified many Wnt signaling components and have revealed their underlying mechanisms. However, important gaps remain, specifically with respect to the mechanisms of signal transduction regulated by Wnt proteins and β-catenin. For example, although structures of Wnt in complex with the Frizzled CRD have provided details about the mode of binding, it remains unclear how this is coupled to potential allosteric changes in the heptahelical bundles of Frizzled to transduce signaling to the intracellular components. How Wnts co-engage Frizzled and LRP5/6 co-receptors, and the precise nature of the activated complex, also remains poorly characterized. Another loosely understood step is the recruitment of Dishevelled proteins by activated Frizzled receptors, and how this leads to inactivation of destruction complex activity. Furthermore, despite close investigation, the biochemical nature of the destruction complex and the precise molecular mechanisms underlying its function and regulation remain ill defined. The role of β-catenin as a cell-adhesion component when bound to cadherin molecules and whether this pool of β-catenin at the plasma membrane interacts functionally with the transcriptional pool in specific contexts also needs to be evaluated. Similarly, β-catenin transcriptional functions, which are independent of Wnt ligands and influenced by signaling crosstalk with other signaling pathways, need to be considered. In addition, how various levels of Wnt signals are ultimately translated into precise changes in nuclear β-catenin, and hence spatiotemporal modulation of gene expression, is incompletely explained by our current linear view of the Wnt-β-catenin pathway. More work is thus needed to reverse engineer the complex signaling network formed by the 19 Wnt ligands, 10 Frizzled receptors and multiple co-receptors to better understand how, when and where each of these components functions. Finally, elucidating which Wnt-receptor pairs are involved in specific physiological functions will likely provide more selective therapeutic strategies for treating diseases. Advances in cryoelectron microscopy and single-molecule imaging, combined with progress in functional genomics-based approaches, will undoubtedly help us to fill these gaps.

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Competing interests

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