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Focus Review

Mitotic and mitogenic Wnt signalling

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Canonical Wnt signalling plays an important role in development, tissue homeostasis, and cancer. At the cellular level, canonical Wnt signalling acts by regulating cell fate, cell growth, and cell proliferation. With regard to proliferation, there is increasing evidence for a complex interaction between canonical Wnt signalling and the cell cycle. Mitogenic Wnt signalling regulates cell proliferation by promoting G1 phase. In mitosis, components of the Wnt signalling cascade function directly in spindle formation. Moreover, Wnt signalling is strongly activated in mitosis, suggesting that 'mitotic Wnt signalling' plays an important role to orchestrate a cell division program. Here, we review the complex interplay between Wnt signalling and the cell cycle.

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Introduction

The relationship between canonical Wnt signalling and cell proliferation stood at the very beginning of Wnt discovery in mammals 30 years ago. The pioneering work from Nusse and Varmus (1982) identified *Int1* (*Wnt1a*) as the locus associated with mouse mammary tumour virus-driven tumorigenesis. Another milestone was the discovery that cyclin D1 is induced by Wnt signalling, and thereby triggers G1-phase progression and tumour cell proliferation (Shtutman *et al*, 1999; Tetsu and McCormick, 1999).

We now know that there is a complex interplay between cell cycle and Wnt signalling (Figure 1). Wnt signalling regulates G1 progression not only via cyclin D1 but also at multiple levels (Figure 2). Furthermore, Wnt signalling also plays an important role in mitosis (Figures 3 and 4). Consequently, misregulation of Wnt signalling can result in aberrant proliferation and chromosome instability, hallmarks of cancer.

Here, we review the relation between canonical Wnt signalling and the cell cycle in development and disease. We discuss the role of mitogenic Wnt signalling in G1 progression, self-renewal and proliferation of stem cells, as well as the function of 'mitotic Wnt signalling'. Excellent reviews dealing with other aspects of Wnt signalling, including the mechanism of canonical Wnt signalling and its role in stem-cell biology and disease are available (Bienz and Clevers, 2000; Reya and Clevers, 2005; Clevers, 2006; MacDonald *et al*, 2009; van Amerongen and Nusse, 2009; Niehrs and Acebron, 2010; Kikuchi *et al*, 2011; Metcalfe and Bienz, 2011).

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Wnt signalling and G1 phase

In order to divide without losing mass and genetic information, cells have to grow and replicate their DNA before division. Cells compartmentalize these processes in consecutive steps, which compose the cell cycle (Figure 1). In G1 and G2 phases, cell growth and transcription occur. DNA is replicated in S phase, while chromosomes are condensed and segregated after G2, during mitosis. Progression through the cell cycle is regulated by checkpoints that sense defects related to the different phases, in particular errors associated with genomic integrity. These checkpoints are modulated by cyclins and their cyclin-dependent kinases (CDKs), which integrate this information and switch between cell-cycle progression and arrest (Malumbres and Barbacid, 2009; Figure 1). Halting at a checkpoint allows cells to repair defects, such as errors in DNA replication or chromosome segregation. If damage is too severe, then cells may undergo apoptosis. On the other hand, mitogenic signals promote progression at checkpoints and thereby cell proliferation. Misregulation of checkpoints can compromise cell homeostasis and lead to both unscheduled proliferation and accumulation of DNA damage (Malumbres and Barbacid, 2009).

Entering S phase and DNA replication is a key decision that typically forces cells to divide, and hence this decision has to be regulated during G1. Indeed, most signalling pathways that regulate cell proliferation exert their effects in G1 (Massague, 2004). Thus, the balance of signals during this phase needs to be tightly coordinated and not surprisingly misregulations of such pathways are frequently associated with disease, notably cancer (Massague, 2004). Two important checkpoints for the G1 to S progression are regulated by cyclin D, cyclin E and their CDKs (Figure 1). Cyclin D accumulates and regulates many G1 events (Baldin *et al*, 1993; Malumbres and Barbacid, 2009), including phosphorylation and inhibition of the Retinoblastoma (Rb) complex, which increases cyclin E levels, whose accumulation acts as G1/S checkpoint (Dulic *et al*, 1992). S phase is then initiated after cyclin E degradation. Under growth inhibitory conditions such as serum starvation or TGFβ signalling, p21 and p27 accumulate and inhibit cyclin D and E, thereby leading to cell-cycle exit and entry into

quiescence (G_0) (Toyoshima and Hunter, 1994; Robson *et al*, 1999). Misregulation of cyclins and their CDKs induces unscheduled proliferation and is commonly associated with cancer (Massague, 2004; Malumbres and Barbacid, 2009).

Canonical Wnt signalling (Box 1) plays a key mitogenic role in promoting G1 progression by inhibiting GSK3, which directly regulates β-catenin-dependent *c-myc* transcription, cell-cycle effectors, and growth regulators.

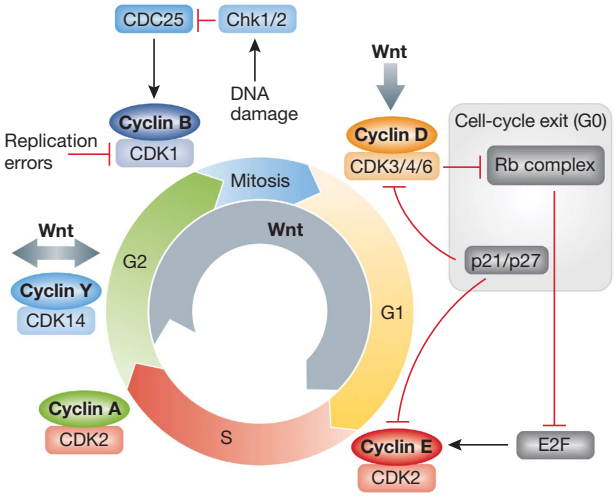

Figure 1 Overview of Wnt in cell-cycle regulation. Cell-cycle progression is controlled by cyclins and their CDKs. In G1, cyclin D initiates Rb complex phosphorylation, which derepresses E2F to induce *cyclin E* transcription. Components of this complex as well as p21 and p27 oppose these effects and can result in cell-cycle exit to G_0. After DNA replication in S phase, different quality controls ensure the integrity of the DNA, while a cyclin B/CDK1 complex orchestrates progression into mitosis. Chromosome abnormalities and DNA damage are reported to this complex via different pathways to delay or stop cell division. Canonical Wnt signalling can regulate the cell cycle at the indicated levels, further described in the text.

β-*Catenin-dependent c-myc regulation*

Possibly the most important mechanism whereby Wnt signalling promotes G1 progression, is by inducing *c-myc*, which is a direct target gene of β-catenin (He *et al*, 1998). *C-myc* encodes a transcription factor that has a dual role in G1 progression, upregulating *cyclin D* (Daksis *et al*, 1994) and repressing *p21* and *p27* (Figure 2; Gartel *et al*, 2001; Yang *et al*, 2001; van de Wetering *et al*, 2002). Aberrant cyclin D1 and c-myc upregulation takes place when β-catenin is hyperactivated (Tetsu and McCormick, 1999; van de Wetering

Box 1: Canonical Wnt signalling overview

The essence of canonical Wnt signalling is a cascade of events, which leads to the inhibition of glycogen synthase kinase 3 (GSK3), which regulates many substrates, notably β-catenin. Phosphorylation by GSK3 triggers degradation of many of its substrates, including β-catenin via the proteasome (Aberle *et al*, 1997) and Wnt signalling stabilizes such proteins (Taelman *et al*, 2010). In canonical Wnt signaling, Wnt ligands form a ternary complex with Frizzled (Fzd) and LRP6 coreceptors (Wodarz and Nusse, 1998; Tamai *et al*, 2000; Wehrli *et al*, 2000). Upon Wnt ligand binding, the signalling complex forms clusters with Dishevelled (Dvl) polymers, to form endocytic LRP6 signalosomes (Bilic *et al*, 2007; Schwarz-Romond *et al*, 2007; Cruciat *et al*, 2010). LRP6 signalosomes promote phosphorylation of LRP6, which is required for signal transduction. So-called PPPSP motifs in LRP6 are phosphorylated in a Wnt-dependent as well as independent manner by a variety of kinases including GSK3 (Zeng *et al*, 2005) and cyclinY/CDK14 (Davidson *et al*, 2009). Phosphorylation of PPPSP motifs primes CK1γ to phosphorylate the adjacent Ser/Thr cluster upon Wnt stimulation (Davidson *et al*, 2005; Zeng *et al*, 2005). LRP6 phosphorylation by CK1γ triggers recruitment of GSK3 and other components of the β-catenin destruction complex to the signalosomes, notably APC and Axin (Bilic *et al*, 2007). Thereby, β-catenin is derepressed, accumulates and enters the nucleus to initiate transcription of target genes (listed in http://www.stanford.edu/~rnusse/wntwindow.html). LRP6 signalosomes mature into multivesicular bodies, where GSK3 is sequestered and its inhibition is sustained (Taelman *et al*, 2010).

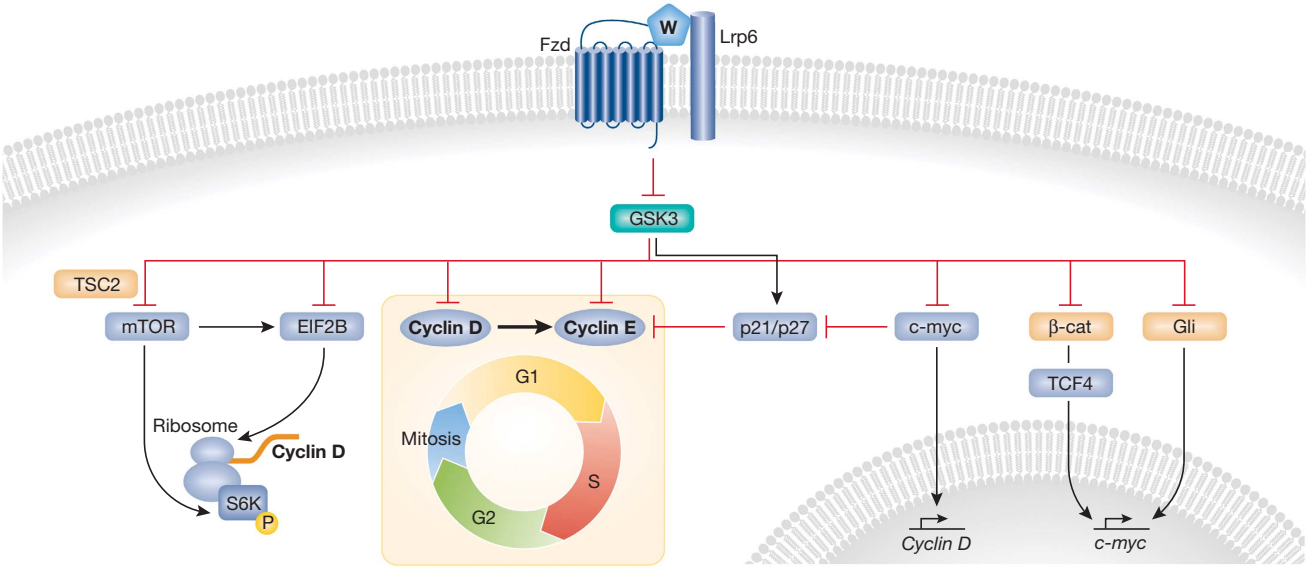

Figure 2 Regulation of G1- to S-phase progression by Wnt signalling. GSK3 inhibition by Wnt signalling acts as a central node for G1 control. GSK3 inhibits or activates the indicated proteins, all of which can contribute to G1- to S-phase progression. GSK3 target proteins known to be regulated by Wnt ligands are indicated in ochre.

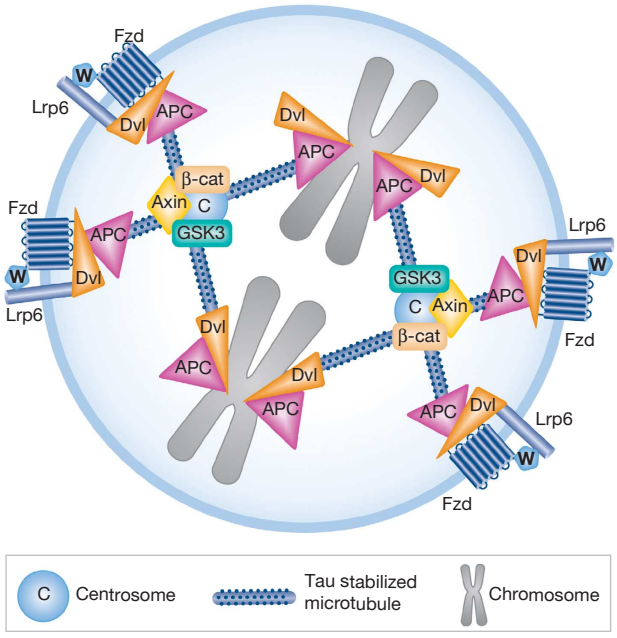

Figure 3 Mitotic spindle regulation by Wnt signalling components. APC and Dvl regulate the attachment of the mitotic spindle to the kinetochores, and together with Fzd and LRP6 modulate spindle orientation. GSK3, β-catenin, and Axin2 are required at the centrosome to ensure a proper distribution of the chromosomes during division. Wnt/GSK3 signalling promotes microtubule assembly by tau stabilization. Inhibition of Wnt signalling or mutations in the indicated components compromise the mitotic spindle and can result in chromosome instability.

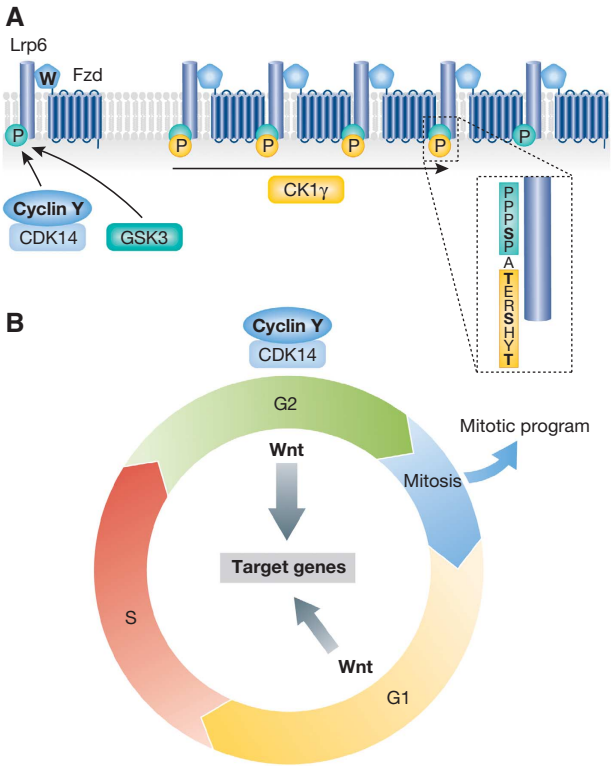

Figure 4 Mitotic Wnt signalling. (**A**) LRP6 competence depends on PPPSP sites phosphorylation (green) by GSK3 or the G2/M cyclinY/CDK14 complex, which primes CK1γ to further phosphorylate LRP6 upon Wnt stimulation (yellow). The architecture of the Lrp6 phosphorylation motif A is highlighted as an example, further described in Box 1. (**B**) Phosphorylation of the Wnt coreceptor LRP6 by cyclin Y/CDK14 results in maximal Wnt signalling at G2/M. Whether activation of LRP6 impacts the mitotic program through Wnt signalling components is still unknown.

et al, 2002). This occurs in most colorectal cancers, where mutations of adenomatous polyposis coli (APC), a component of the β-catenin destruction complex, lead to a permissive and fast G1 transition and neoplastic transformation (Arber *et al*, 1997; Morin *et al*, 1997; Tetsu and McCormick, 1999).

Stabilization of cell-cycle effectors

Among the substrates which are destabilized by GSK3 phosphorylation are also direct regulators of G1- to S-phase progression, notably cyclin D1, cyclin E1 and c-myc (Diehl *et al*, 1998; Welcker *et al*, 2003, 2004; Figure 2). Mutations that impair phosphorylation and proteolysis of these G1–S regulators are often associated with neoplasia (Welcker *et al*, 2004). Thus, Wnt signalling-mediated GSK3 inhibition not only induces transcription of G1 regulators such as c-myc and cyclin D1 via β-catenin, but may also directly increase their protein stability. Another example is Wnt crosstalk with the sonic hedgehog pathway via GSK3, which phosphorylates Gli/Ci (Borycki *et al*, 2000; Price and Kalderon, 2002). By inhibiting GSK3, Wnt signalling stabilizes the transcription factor Gli (Borycki *et al*, 2000), which promotes expression of the G1 regulator N-Myc (akin to c-myc) (Mill *et al*, 2005).

Regulation of cell growth

When cells divide, they need to increase their protein levels, otherwise they will become smaller. Most cellular growth occurs in G1 and different mitogenic growth signals converge in this phase (Massague, 2004). They synchronize cell-cycle progression and cell mass in order to keep cell size constant. GSK3 is a key inhibitor of cell growth, and this inhibition is released by growth factors like IGF acting through AKT kinase, which blocks GSK3, resulting in an increased glucose uptake and TOR pathway activation (Sutherland *et al*, 1993). Notably, the TOR pathway is a key regulator of G1 growth by activating protein synthesis.

Similarly to IGF/AKT, Wnt/GSK3 signalling also activates the TOR pathway to stimulate protein translation (Inoki *et al*, 2006). Wnt activation has a dual role in the TOR pathway because GSK3 directly destabilizes EIF2B, a TOR pathway effector (Welsh *et al*, 1998), and activates TSC2, an inhibitor of the TOR pathway (Inoki *et al*, 2006; Figure 2). Interestingly, this TOR activation is yet another mode whereby Wnt signalling increases cyclin D1 protein, because *cyclin D1* mRNA translation highly depends on the TOR pathway (Inoki *et al*, 2006). Thereby Wnt signalling coordinates growth and cell-cycle progression in G1 with GSK3 as its nexus, by directly regulating GSK3 substrates, independent of β-catenin-mediated transcription.

The tight link between Wnt signalling and G1 progression has important consequences in the context of development. Wnt-dependent c-myc/Cyclin D accumulation regulates cell proliferation and thereby patterning during embryogenesis. For example, during spinal cord development Wnts form a dorsal to ventral proliferation gradient (Megason and McMahon, 2002). Another example are developing long bones where Wnt signalling promotes chondrocyte

proliferation and longitudinal growth (Yang *et al*, 2003). In adults, mitogenic Wnt signalling promotes cell turnover during tissue homeostasis or following injury (Stoick-Cooper *et al*, 2007b; Blanpain and Fuchs, 2009; Haegebarth and Clevers, 2009; Minear *et al*, 2010; Rabbani *et al*, 2011). The balance between quiescence and proliferation must be fine-tuned in order to avoid either a fatal loss of the tissue regeneration or neoplasia (Flores *et al*, 2004; Massague, 2004; Chen *et al*, 2009; Haegebarth and Clevers, 2009). The dissection of the various modes whereby Wnt signalling impacts G1 may help designing specific cancer therapies.

Wnt signalling and mitosis

The major role whereby Wnt signalling is commonly thought to impact the cell cycle is by transcriptional and translational upregulation of G1 effectors. However, it has become clear that another important phase where Wnt signalling plays a key role is mitosis. In mitosis, cells divide not only chromosomes but also all cellular constituents into daughter cells. Mitosis is therefore predominantly a phase of subcellular mechanics, while transcription and translation are mostly inhibited (Prescott and Bender, 1962; Gottesfeld and Forbes, 1997). Mitotic entry is initiated at late G2 by the cyclin B/CDK1 complex (Takizawa and Morgan, 2000), which is a checkpoint that is only activated when sufficient cell growth has occurred and DNA has been replicated (Malumbres and Barbacid, 2009; Figure 1). A protein network triggered by cyclin B/CDK1 induces nuclear envelope breakdown, chromosome condensation, and centrosome separation (Minshull *et al*, 1989; Takizawa and Morgan, 2000; Jackman *et al*, 2003). Microtubules (MTs) project from opposing centrosomes and attach to kinetochores to form the mitotic spindle. When the chromosomes are properly aligned, the Anaphase-Promoting Complex (APC/C; not to be confused with APC) degrades cyclin B and leads to chromosome segregation into daughter cells (Irniger *et al*, 1995; Zachariae *et al*, 1998). DNA replication errors trigger the inhibition of the cyclin B/CDK1 complex, for example, via Chk1/2 (Sanchez *et al*, 1997), and impair entry in mitosis, unless repaired (Figure 1). Once mitosis is initiated several positive feedback loops, notably via CDC25 (Sanchez *et al*, 1997; Takizawa and Morgan, 2000), reinforce commitment to the mitotic spindle formation. DNA integrity is then monitored by the spindle checkpoint, which inhibits APC/C until the sister chromatids are aligned without abnormalities, to avoid chromosome instability (Michel *et al*, 2001; Reddy *et al*, 2007).

Wnt signalling components regulate mitosis

Components of the Wnt signalling pathway modulate different aspects of the mitotic program, including MT dynamics, spindle formation, and centrosome division.

MT dynamics are regulated by Wnt signalling, and not only in mitosis (Salinas, 2007). In mitosis, long-term GSK3 inhibition by LiCl can abolish MT growth, as do stabilizing mutations in β-catenin or Axin2 ablation, while short-time GSK3 inhibition accelerates the reorganization of MTs (Huang *et al*, 2007). This may reduce phosphorylation of MT-associated proteins, such as the BICD–dynein complex and tau (Hanger *et al*, 1992; Fumoto *et al*, 2006), which stabilize MTs and the mitotic spindle (Wakefield *et al*, 2003; Fumoto *et al*, 2006). Consequently, GSK3 inhibitors can induce chromosome instability (Tighe *et al*, 2007).

Axin2 and APC localize to the mitotic spindle and are required for chromosome segregation (Fodde *et al*, 2001; Hadjihannas *et al*, 2006; Figure 3). APC associates with the MT plus ends and connects them to kinetochores, which is essential for chromosome organization and segregation. Mutations in *APC* that are associated with chromosome instability in colon cancer (Fodde *et al*, 2001; Hadjihannas *et al*, 2006) impair the linkage between MTs and kinetochores (Fodde *et al*, 2001) and upregulate *Axin2*, compromising the mitotic spindle checkpoint (Hadjihannas *et al*, 2006). Similarly, Dvl cooperates with Plk1 in establishing the spindle orientation and this depends on Fzd and LRP6 coreceptors (Kikuchi *et al*, 2010; Figure 3).

Centrosomes, which align the mitotic spindle, are another hot spot for Wnt components. Axin2, GSK3, and β-catenin accumulate at the centrosomes, where they regulate MT growth. For example, β-catenin depletion or LiCl treatment cause disruption of radial MTs (Huang *et al*, 2007). Moreover, stabilizing mutations in β-catenin or Axin2 ablation lead to premature splitting of the centrosome or multiple centrosomes (Huang *et al*, 2007; Bahmanyar *et al*, 2008; Hadjihannas *et al*, 2010). Finally, GSK3 may also play a role in the curious phenomenon whereby proteins destined for proteasomal degradation localize preferentially to only one of the two centrosomes during mitosis. This leads to asymmetric protein distribution, even in otherwise 'non-polarized' cells (Fuentealba *et al*, 2008). Thus, Wnt pathway components organize various centrosomal functions in mitosis.

While it is well established that multiple Wnt signalling components play an important role during mitosis, there is little evidence that these processes are actually regulated by Wnt signalling. The function of Dvl and APC at the mitotic spindle requires LRP6 and Fzd (Kikuchi *et al*, 2010) but an involvement of Wnt ligand signalling has not been demonstrated and is an important question that needs to be addressed.

Mitotic Wnt signalling

An unexpected finding was that Wnt signalling not only impacts the cell cycle, but that the reverse is also true, specifically that G2/M is a privileged phase for Wnt signalling. This is because the competence of the Wnt coreceptor LRP6 to respond to Wnt ligands is maximal during G2/M. The competence of LRP6 for Wnt signalling depends on a priming phosphorylation of its PPPSP sites (Box 1). The cyclin-dependent kinase 14 (CDK14/PFTK1) phosphorylates the LRP6 PPPSP motifs and associates with and is regulated by the G2/M cyclin Y. As a consequence, LRP6 phosphorylation peaks during G2/M and thus the coreceptor is maximally primed to respond to incoming Wnt signals (Davidson *et al*, 2009; Figure 4A and B). This explains previous observations that cytoplasmic β-catenin levels oscillate with the cell cycle, peaking in mitosis (Orford *et al*, 1999; Olmeda *et al*, 2003). Interestingly, IGF signalling also peaks at G2/M (Shtivelman *et al*, 2002), suggesting a coordinated cell-cycle modulation of mitogenic signals, notably since both pathways converge in GSK3 regulation.

Recently, it has been confirmed that expression of the Wnt/β-catenin target genes *Lgr5* and *Axin2* peaks at G2/M. Intriguingly, other Wnt targets like *c-myc* peaked at G1/S

(Hadjihannas *et al*, 2012). Differences in peak target gene expression may be due to crosstalk with other signalling pathways as well as to a negative feedback loop promoted by Axin2 (Davidson *et al*, 2009; Hadjihannas *et al*, 2012).

Wnt signalling and cell-cycle regulation in stem cells

Wnt signalling has a prominent role in stem cell biology, including self-renewal, pluripotency, and differentiation of both embryonic stem (ES) and somatic stem cells (reviewed in Reya and Clevers, 2005; Clevers, 2006; Nusse *et al*, 2008; Sokol, 2011).

Wnt regulation of ES cells

Pluripotency of ES cells is maintained by a core network consisting of Oct4, Sox2, and Nanog (Pan and Thomson, 2007). In mouse ES cells, Wnt signalling plays a key role in the maintenance of pluripotency and transcriptional regulation of this core network (Kielman *et al*, 2002; Sato *et al*, 2004; Hao *et al*, 2006; Ogawa *et al*, 2006; Miyabayashi *et al*, 2007; ten Berge *et al*, 2011). In a dual mode, β-catenin forms a complex with Tcf1 to upregulate *Oct4* transcription (Sato *et al*, 2004; ten Berge *et al*, 2011) and blocks Tcf3 (Cole *et al*, 2008; Kelly *et al*, 2011; Wray *et al*, 2011), which is an inhibitor of the core network (Cole *et al*, 2008). The effect of Wnt/β-catenin signalling on maintaining core network expression is fully accounted for by Tcf3 repression and Tcf1-dependent transcription (Niwa, 2011; Yi *et al*, 2011). Although β-catenin is required for the self-renewal promoted by Wnt/GSK3 signalling (Kelly *et al*, 2011; Niwa, 2011; Wray *et al*, 2011; Yi *et al*, 2011), it is not essential for mouse ES cell self-renewal under specific culture conditions (Lyashenko *et al*, 2011; Wray *et al*, 2011).

Despite the prominent role of canonical Wnt signalling in ES cells self-renewal, surprisingly little is known about whether Wnt signalling regulates ES cell cycle directly. ES cells have a fast cell cycle with a short G1 phase (Becker *et al*, 2006) and progress in the absence of cyclin D (Burdon *et al*, 2002), suggesting that mitogenic Wnt signalling would not impact ES cell-cycle progression via cyclin D1 upregulation. On the other hand, c-myc promotes ES cell self-renewal (Cartwright *et al*, 2005; Ying *et al*, 2008), although its upregulation is not essential for ES cell maintenance under specific culture conditions (Cartwright *et al*, 2005; Ying *et al*, 2008). In ES cells, c-myc forms a regulatory network together with E2F (Chen *et al*, 2008), which upregulates *cyclin E* transcription (Geng *et al*, 1996; Stead *et al*, 2002). Whether Wnt signalling promotes cell-cycle progression in ES cells by c-myc upregulation is a question that remains unsolved.

Wnt regulation of adult stem cells

A hallmark of adult stem cells is their low proliferation, notably in contrast to ES cells, which prevents their exhaustion (Cheng *et al*, 2000; Orford and Scadden, 2008). Quiescence of adult stem cells is maintained by p21 (Cheng *et al*, 2000), which keeps cells arrested in G_0. This is the case in intestinal crypt stem cells, where Wnt signalling promotes cell-cycle entry by inducing *c-myc* and *cyclin D1* expression and downregulating p21 (Korinek *et al*, 1998; Kuhnert *et al*, 2004).

In haematopoietic stem cells (HSCs), Wnt signalling increases both self-renewal and differentiation by *c-myc* induction (Reya *et al*, 2003). Although the essential role of β-catenin in this process was controversial (Cobas *et al*, 2004), recent data suggest that high and low Wnt levels promote differentiation and HSCs self-renewal, respectively (Luis *et al*, 2011).

In hair follicles, Wnt signalling is crucial for both self-renewal and activation of bulge stem cells (Reya and Clevers, 2005; Greco *et al*, 2009). Although the molecular mechanisms underlying these effects are not yet clear, both Wnt/β-catenin and c-myc transcriptional programs overlap at the hair follicle (Choi *et al*, 2008) and both promote proliferation of bulge stem cells (Chan *et al*, 1999; Andl *et al*, 2002; Greco *et al*, 2009). This suggests that Wnt may exert its activities through c-myc by promoting cell-cycle entry. Moreover, different components of the Wnt pathway including GSK3, β-catenin, lef1, and TCF3/4 play distinct roles to coordinate bulge stem-cell differentiation and transit through the follicle (DasGupta and Fuchs, 1999; Nguyen *et al*, 2009; Wu *et al*, 2011).

These examples highlight that Wnt signalling in adult stem cells promotes cell-cycle re-entry by upregulation of c-myc/cyclin D1 and thereby functions as switch between quiescence and division/differentiation. C-myc has a preeminent role in cell-cycle re-entry and as a common driver of tumorigenesis (Pelengaris *et al*, 2002). Aberrant c-myc activation by Wnt signalling in adult stem cells initiates unscheduled proliferation and neoplasia, associated with cancer in the intestine, haematopoietic lineage, and hair follicle (He *et al*, 1998; van de Wetering *et al*, 2002; Pelengaris *et al*, 2002; Weng *et al*, 2006). Importantly, *c-myc* ablation fully rescues *APC* loss-driven tumorigenesis (Sansom *et al*, 2007), highlighting its importance in mediating the cell proliferation effects of Wnt signalling during tissue regeneration.

Wnt signalling in G_0: quiescent cells

Wnt signalling has been mostly characterized in dividing cells. However, many Wnts are also expressed in differentiated adult tissues, suggesting that Wnt signalling plays important roles in postmitotic cells. Such is the case in neurons, where Wnt signalling regulates axon guidance and neurite outgrowth. In the distal end of axons, Wnt signalling regulates APC association with MTs and thereby induces MT remodelling (Salinas and Zou, 2008). Moreover, Wnt3a and Wnt7a enhance neurite outgrowth via tau derepression (Hall *et al*, 2000; Endo *et al*, 2008). Wnt signalling directly inhibits tau phosphorylation by GSK3 and is therefore β-catenin independent (Hanger *et al*, 1992; Scali *et al*, 2006). Tau phosphorylation is critical for MT dynamics in axons (Drechsel *et al*, 1992), and its misregulation is a hallmark of several neurodegenerative disorders, notably Alzheimer (Grundke-Iqbal *et al*, 1986; Bramblett *et al*, 1993).

In adult liver, APC is essential for zonation, the phenomenon that different metabolic processes are compartmentalized in specialized liver areas (Benhamouche *et al*, 2006). APC is differentially expressed within the liver and restricts Wnt/β-catenin signalling to regions where its protein levels are low. In these zones, Wnt signalling activates ammonia metabolism-associated genes, and thereby promotes hepatocyte specialization (Benhamouche *et al*, 2006). Notably, the role of Wnt/β-catenin signalling in liver zonation does not require upregulation of cell-cycle effectors (Burke *et al*, 2009).

In postmitotic cardiomyocytes, components of Wnt signalling regulate cell growth and are notably involved in cardiac hypertrophy, an enlargement which can lead to heart failure (ter Horst *et al*, 2012). Inhibition of GSK3, overexpression of Dvl, and stabilization of β-catenin all lead to aberrant cell growth and heart hypertrophy (Haq *et al*, 2003; Malekar *et al*, 2010). This occurs through many different modes that include TOR activation, NFAT upregulation, and transcription of β-catenin target genes (Blankesteijn *et al*, 2008; ter Horst *et al*, 2012). While there is evidence for a role of Fzd2 in promoting hypertrophy (Blankesteijn *et al*, 2008), it remains unclear whether Wnt ligands or only downstream components are involved in cardiac hypertrophy (Haq *et al*, 2003).

Concluding remarks

Three decades ago, it was discovered that Wnt signalling is associated with deregulated proliferation and neoplasia. Since then key roles of the canonical pathway in cell-cycle regulation have been well established and the underlying mechanisms are emerging. But these studies have also raised new questions that need to be addressed.

What are the targets of β-catenin-independent canonical Wnt signalling?

While canonical Wnt signalling is often equated with β-catenin-dependent transcriptional regulation of target genes, such as *c-myc* and *cyclin D1*, it is becoming increasingly clear that this is only one of the modes whereby the pathway regulates the cell cycle. The other major mode is by inhibiting GSK3, a key kinase with pleiotropic effects and a multitude of substrates, which are typically negatively regulated by GSK3. Recent data from De Robertis and colleagues indicate that a significant part of the proteome is stabilized by Wnt signalling halting GSK3-driven proteolysis (Taelman *et al*, 2010). Bioinformatic analysis of putative GSK3 substrates revealed that many of them function in mitosis, cell cycle, chromatin, and ribosome biogenesis (Taelman *et al*, 2010), suggesting a largely unexplored layer of cell-cycle regulation by Wnt signalling.

What is the physiological significance of 'mitotic Wnt signalling'?

The fact that Wnt signalling peaks in mitotic cells and conversely that Wnt components play such an important role in mitosis suggests that Wnt may orchestrate a mitotic program. What role does Wnt-dependent signalling play in regulating mitotic Wnt components? Dvl, APC, Axin2, and β-catenin all play important roles in spindle mechanics. Yet, whether their functions are Wnt regulated is unresolved.

What is the role of Wnt signalling in quiescent cells?

While most work on Wnt signalling has focussed on dividing cells, the examples of neurons, cardiomyocytes, and hepatocytes indicate important cellular roles in postmitotic tissues. Yet, we still know little about Wnt signalling in quiescent cells. Are there common themes? Individual components of the Wnt pathway have both Wnt-dependent and Wnt-independent functions. Is the requirement for Wnt components in quiescent cells reflecting actual Wnt ligand signalling, or do these components function Wnt independently? This could be addressed, for example, by overexpression of Wnt antagonists such as Dkk1 or studying LRP6 knockout mice.

What is the basis for tissue-specific involvement of Wnt signalling in cancer?

Deregulation of Wnt signalling has been well documented as the basis for a limited number of tumours. Yet, Wnt signalling occurs much more widespread, but without apparent association with neoplasia. What determines this specificity? Crosstalk with other mitogenic pathways likely plays a role and hence it will be important to define these.

How can we exploit Wnt cell-cycle regulation in regenerative medicine and cancer therapy?

Wnt signalling is a prime target in cancer treatment (Barker and Clevers, 2006) and interestingly not just pathway inhibition but also activation may cure certain cancer types (Biechele *et al*, 2012). Given the many roles of Wnt signaling in adult tissues, it appears crucial to identify specific targets to manipulate the pathway in cancer in order to achieve the desired therapy without deleterious side effects. Similarly, in regenerative medicine, specific modulation of Wnt signaling holds great promises (Stoick-Cooper *et al*, 2007a) but again specificity and control are essential, notably in light of the tumorigenic effects of the pathway.

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Conflict of interest

The authors declare that they have no conflict of interest.

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