Regulation of cell cycle dynamics and mitosis by Wnt signaling

Abstract

The cell cycle and cell division are processes that need to be highly controlled in order for organisms to function and to prevent diseases. Cell behavior is controlled through many different signaling pathways, wherein external signals are internalized and trigger a cascade of signaling events within the cell, resulting in a cellular response. The Wnt signaling pathway is an important regulator of cell proliferation, tissue development and homeostasis. Wnt signaling can mainly regulate cell behavior by controlling target gene transcription. However, Wnt signaling can also directly regulate the microtubule cytoskeleton. It is becoming increasingly clearer that Wnt signaling can affect the cell cycle and mitosis at various points. While Wnt signaling controls G1/S phase progression, the cell cycle can control the amplitude of Wnt signaling in G2/M phase, highlighting a bidirectional relationship between Wnt signaling and the cell cycle. Furthermore, Wnt signaling is an important regulator of cell size during interphase and plays numerous roles during mitosis and cell division. In this review, we first discuss the complexity of Wnt signaling by highlighting the different Wnt signaling pathways. Next, we discuss the effects of Wnt signaling on the cell cycle, and vice versa. Lastly, we discuss how Wnt signaling controls mitotic progression, and highlight a role of Wnt signaling in abscission, the last stage of cell division.

Karen van den Anker	***Supervisor and Examiner***	***Second Examiner***
(0847461) - CSND	Dr. Agathe Chaigne	Dr. Frederik Verweij

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Lay summary

Cell division is a crucial process for organisms to not only develop, but also grow and function. During the cell cycle the chromosomes are first duplicated, and then the cell is divided into two new daughter cells, each containing an identical set of genetic material. Understanding how cells control the cell cycle is not only key to understanding how we grow and develop, but also to understanding diseases. Cell proliferation, the growth and subsequent duplication of cells, can be regulated by external cues, such as signal molecules. These signals are then internalized by the cell, and result in a cellular effect which can instruct the cells behavior. One of these pathways is the Wnt signaling pathway. Wnt proteins are growth factors which promote, amongst other things, cell proliferation. Wnt signaling can regulate cell behavior via different pathways. These different pathways can be categorized as β-catenin dependent and β-catenin independent. The β-catenin dependent pathway (also known as the canonical Wnt signaling pathway) relies on the stabilization of the cytoplasmic protein β-catenin. This protein can then go to the nucleus where it serves as an activator of genes important for cell proliferation. There are many β-catenin independent Wnt signaling pathways (non-canonical Wnt signaling pathways). These pathways do not rely on the stabilization of β-catenin, and they are involved in the polarization (asymmetry) of cells, and they can directly influence the microtubule network. Microtubules are long, hollow, cylindrical polymers that are important for intracellular trafficking and during mitosis (division of genetic material). During mitosis, the microtubule network forms a spindle arising from two opposite poles of the cell, which is important for the separation of chromosomes. Interestingly, research has shown that Wnt signaling can control the cell cycle and vice versa. Moreover, Wnt signaling can directly control the mitotic spindle, thus plays a role in mitosis.

In this review, we first discuss the different pathways through which Wnt signaling can regulate cell behavior. Next, we discuss how Wnt signaling controls the progression from one phase of the cell cycle to the next; we discuss how Wnt signaling can control the mitotic spindle, and how errors in Wnt signaling results in improper chromosome segregation. Lastly, we highlight a role of Wnt signaling in the last phase of cell division, in which the membrane connecting the two daughter cells is cut.

Introduction

The cell cycle is a highly fundamental process that ensures the duplication of genetic material and the subsequent distribution of genetic material to daughter cells through cell division. Tight regulation of these processes is essential for the development, maintenance, growth, and repair upon injuries of organisms. Understanding the mechanisms that regulate the cell cycle and mitosis is crucial for unraveling not only the processes involved in growth and development, but also those underlying disease. Cell proliferation is highly coordinated by signaling pathways. One of these signaling pathways regulating in cell proliferation is the Wnt signaling pathway. Wnt proteins are growth stimulatory factors that regulate the cells behavior trough different pathways. Activation of the Wnt signaling pathway can lead to the stabilization of the cytoplasmic protein β-catenin which regulates gene transcription (canonical signaling). Moreover, Wnt signaling, independently of β-catenin, can directly interact with the cytoskeleton to regulate cell shape and polarization or regulate cell polarity (Nusse & Clevers, 2017). Furthermore, many other proteins beside β-catenin get stabilized upon Wnt pathway activation (Wnt/STOP pathway), which plays a major role in cell size regulation.

Although the first member of the Wnt protein family was first discovered over 40 years ago (Nusse & Varmus, 1982), the exact mechanisms through which Wnt signaling regulates cell proliferation is yet to be completely understood. Interestingly, the regulation of cell proliferation through Wnt signaling is coordinated by the ability of Wnt signaling to impact the cell cycle at various points (Niehrs & Acebron, 2012). Moreover, it has been shown that levels of cytoplasmic β-catenin and Axin-2, a downstream target of Wnt signaling, oscillate through the cell cycle and peak at G2/M (Olmeda et al., 2003), highlighting there is a complex interplay between Wnt signaling and the cell cycle and mitosis. Indeed, mis-regulation of Wnt signaling can result in chromosomal instability and cytokinesis defects (Fumoto et al., 2012; Hadjihannas et al., 2006).

Recently it has become progressively clearer that both β-catenin dependent and independent Wnt signaling play a role in cell cycle regulation and mitosis. Here, we first discuss the many facets of Wnt signaling. Next, we discuss how Wnt signaling pathway components are involved in cell cycle progression, and how the cell cycle regulates Wnt signaling, focusing on animal cells. Lastly, we highlight interactions of the Wnt signaling with the cell division machinery.

1. The many facets of Wnt signaling

The Wingless/integrase-1 (Wnt) signaling pathway plays an important role during development and homeostasis across different species, including mammals, *C. elegans*, *Drosophila* and *Xenopus*. Wnt proteins are secreted growth-stimulatory factors which regulate, amongst others, cell proliferation, differentiation, polarity, and migration (Nusse & Clevers, 2017; M. Yu et al., 2023). The human genome encodes for 19 different Wnt isoforms, having either unique or partially overlapping functions as shown by various loss of functions studies (web.stanford.edu/group/-nusselab/cgi-bin/wnt). Wnt ligands, which are 40 kDa in size, undergo lipid modification during synthesis in the endoplasmic reticulum (ER) (Takada et al., 2006; Willert et al., 2003). This involves the attachment of a lipid, an acyl group termed palmitoleic acid, by a membrane-bound O-acyltransferase, Porcupine, on a conserved serine group in the Wnt protein. This palmitoylation is necessary for the binding of Wnt to a cargo receptor called Wntless/Evi (WLS), which is involved in the transportation of Wnt from the ER to the plasma membrane. Moreover, this lipid modification functions as a binding motif for the Wnt receptor frizzled (Fzd) (reviewed elsewhere in: Nusse & Clevers, 2017).

Wnt signaling can act through multiple distinct pathways, each resulting in specific downstream effects. These distinct pathways are all initiated by the binding of Wnt ligands a receptor complex consisting of the Fzd receptor and a pathway specific co-receptor (Acebron et al., 2014). Fzd is a seven pass-transmembrane receptors which contains an extracellular cystine rich domain (CRD). The c-terminus of Wnt interacts with this CRD, which is important for binding of Wnt to Fzd receptor. While Fzd serves as the primary receptor for Wnt ligands, the specificity of Wnt signaling is controlled by the distinct co-receptors. Among these co-receptors, which can be categorized in 6 different protein families, are the low-density lipoprotein receptor-related protein 5 and 6 (LRP5 and LRP6), protein Tyr kinase 7 (PTK7), receptor Tyr kinase (RYK), and receptor Tyr kinase-like orphan receptor 1 and 2 (ROR1 and ROR2) (Niehrs, 2012).

Wnt signaling can be divided in the canonical (or β-catenin) pathway (Nusse & Clevers, 2017), the Wnt/PCP pathway, the Wnt/Ca^{2+} pathway (M. Yu et al., 2023), and the lesser known Wnt/Stabilization of proteins (Wnt/STOP) pathway (Acebron et al., 2014; Y. L. Huang et al., 2015; Lin et al., 2021). The canonical Wnt signaling pathway, and Wnt/STOP both depend on the stabilization of β-catenin and are initiated by the binding of Wnt to the Fzd/Lrp5/6 receptor complex. The Wnt/PCP pathway is initiated by binding of Wnt to Fzd and PTK7, and Wnt/Ca2+ signaling by ROR1/2. While canonical Wnt signaling primarily regulates gene expression via β-catenin to induce for instance cell proliferation, non-canonical Wnt signaling functions independently of β-catenin and is involved in cell polarization and migration (M. Yu et al., 2023).

1.1. The canonical Wnt signaling pathway

The canonical Wnt signaling pathway plays an important role in not only development, but also in tissue homeostasis and cancer. The canonical Wnt signaling pathway is dependent on the transcriptional regulator β-catenin. In the absence of Wnt ligands, β-catenin is marked for degradation by a so-called destruction complex (DC). This destruction complex consists of the tumor suppressor Adenomatous polyposis coli (APC), two kinases, Casein kinase 1α (CK1α) and Glycogen synthase kinase-3 beta (GSK-3β), and the scaffolding protein Axin (Figure 1A). In this complex, Axin interacts with the different

components of the DC and with β-catenin. Then, the two kinases, CK1α and GSK-3β, sequentially phosphorylate β-catenin creating a degron motif. Next, E3 ubiquitin ligases, such as β-TrCP, recognize these degron motifs and mark β-catenin for proteasomal degradation. In the presence of Wnt, these ligands bind to their Fzd receptor and their co-receptor LRP5/6 (Figure 1B) (Tamai et al., 2000; Wehrli et al., 2000). This complex subsequently recruits the protein Disheveled (Dvl), which phosphorylates the cytoplasmic tails of the LRP5/6 co-receptor. After phosphorylation, LRP5/6 binds Axin, thereby deconstructing the degradation complex and releasing β-catenin of proteasomal degradation. Accumulated β-catenin translocates to the nucleus, where it replaces the repressor Groucho and binds to transcription factors of the T-cell/lymphoid enhancer factor (TCF/LEF) family. Together this forms a complex that regulates the transcription of target genes involved in various cellular processes, such as cell proliferation (reviewed elsewhere in Cadigan & Waterman, 2012; Nusse & Clevers, 2017; Yu et al., 2023).

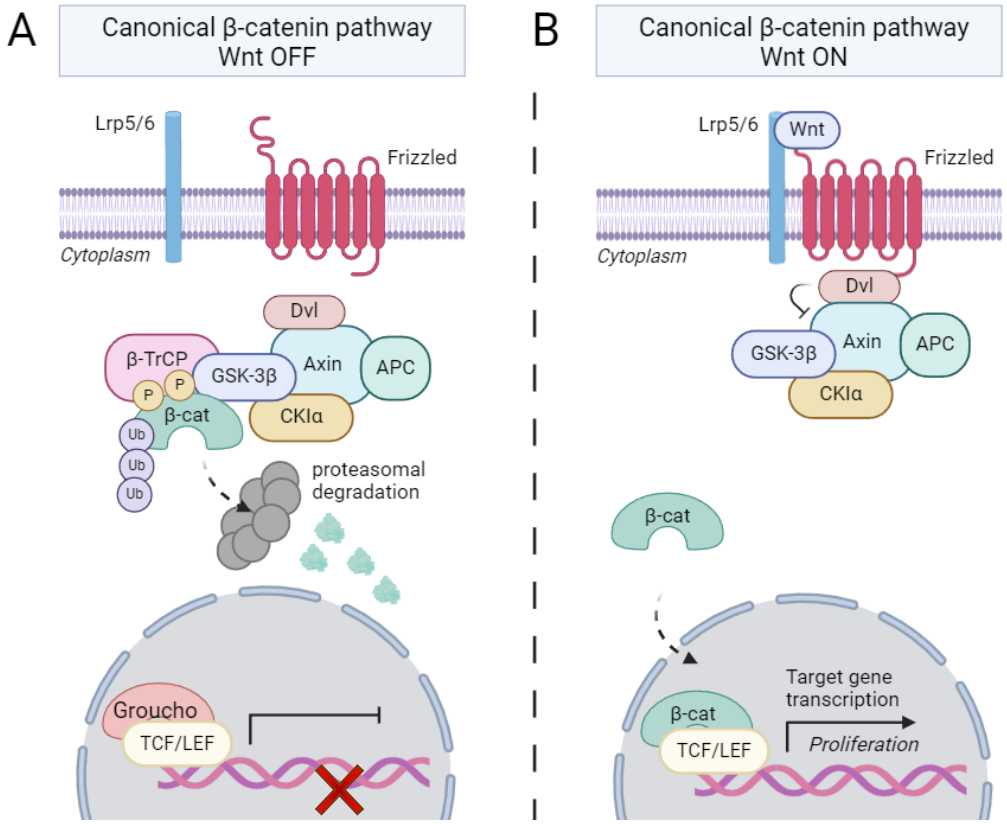


Figure 1: Simplified canonical Wnt signaling
(A) *In the absence of Wnt ligands, β-catenin is phosphorylated by the destruction complex consisting of the proteins Dvl, Axin, APC, CK1α, GSK-3β. Subsequently the E3-ubuiqitin ligase recognizes the phosphorylated β-catenin and marks it for proteasomal degradation through poly-ubiquitination.* ***(B)*** *In the presence of Wnt ligands, Dvl induces the interaction of Axin and LRP5/6 through phosphorylation of the cytoplasmic tail of LRP5/6. This results in the deconstruction of the DC, and the stabilization of β-catenin. β-catenin can then translocate to the nucleus, where it induces gene transcription. Figure created with Biorender.*

1.2. The non-canonical Wnt signaling pathways

The **Wnt/planar cell polarity (PCP) pathway** regulates the establishment of polarity within for example epithelial tissues and influences processes such as cell migration and tissue morphogenesis. In the Wnt/PCP pathway, Wnt ligands bind the Fzd receptor as well as co-receptors such as PTK7. The binding

of Wnt to Fzd results in phosphorylation of Dvl, which in turn activates small GTPases such as Rac1 and RhoA (Habas et al., 2003). RhoA subsequently activates the Rho-associated protein kinase (ROCK), which is known for its role in actin polymerization, leading to reorganization of the actin cytoskeleton (Peng et al., 2019). Rac1 drives cell motility by promoting the formation of lamellipodia. Rac1 activates c-Jun n-terminal kinases (JNK), which phosphorylate c-Jun (Coso et al., 1995). c-Jun in turn translocates to the nucleus, where it regulates transcription of genes involved in cell polarity (Meng & Xia, 2011). Together, these modes of action result in cytoskeletal rearrangements important for cell migration and polarity (Figure 2A) (reviewed elsewhere in Yu et al., 2023).

Another branch of non-canonical Wnt signaling is the **Wnt/Ca^{2+} pathway**, which is proposed to play an important role during early embryogenesis. This pathway is important for the regulation of the release of calcium ions from the ER to regulate intracellular calcium levels. This pathway is initiated through binding of Wnt5a to Fzd and the ROR1/2 receptor complex (Grumolato et al., 2010). In turn, Dvl and heterotrimeric G-protein get activated. Together, these proteins activate phospholipase C (PLC) and inositol-1,4,5-trisphosphate (InsP3), in which the latter triggers release of Ca^{2+} from the ER (McQuate et al., 2017). This increase in intracellular calcium levels regulates the transcription of target genes involved in cell migration and cell fate (Figure 2B) (reviewed elsewhere in Yu et al., 2023)

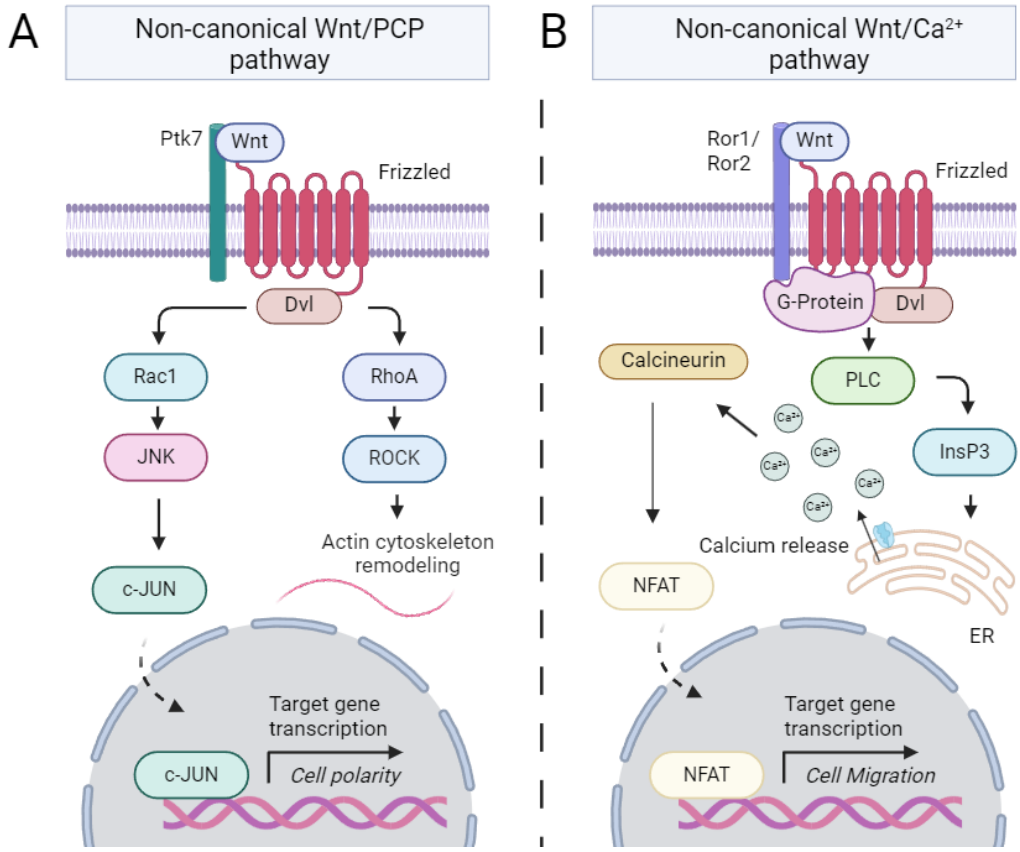


Figure 2: Simplified non-canonical Wnt signaling

(A) *The non-canonical Wnt/PCP signaling pathway. Wnt signaling is transduced by the binding of Wnt to the Fzd/PTK7 receptor complex, resulting in the phosphorylation of Dvl. Dvl in turn activates the small GTPases Rac1 and RhoA which results in actin cytoskeleton remodeling and transcription of genes involved in cell polarity.* ***(B)*** *The non-canonical Wnt/Ca^{2+} signaling pathway is initiated through binding of Wnt5a to the Fzd and ROR1/2 receptor complex. This leads to the recruitment of Dvl and heterotrimeric G-protein, which induces a signaling cascade leading to the release of calcium from the ER. The rise in calcium levels leads to the initiation of transcription of genes involved in cell migration. Figure created with Biorender.*

1.3. Wnt/STOP

Recent developments in the field of Wnt signaling have shown that besides stabilization of β-catenin, Wnt signaling can stabilize other proteins in a GSK-3β dependent matter. Indeed, it has been shown that many other proteins, besides β-catenin, are direct targets of GSK-3β and are regulated by Wnt signaling (Taelman et al., 2010; Xu et al., 2009). This pathway is referred to as the Wnt/mediated stabilization of proteins (Wnt/STOP) (Taelman et al., 2010). Phosphorylation of target proteins by GSK-3β generates degron motifs, which in turn are recognized and targeted for degradation by E3 ubiquitin ligases. Wnt/STOP is regulated by Wnt/LRP6 signaling, which induces the sequestering of the destruction complex in multivesicular bodies, thus inactivating the DC. Therefore, many different proteins besides β-catenin are no longer targeted for degradation, raising the half-life of the cytoplasmic pool of proteins. (Acebron & Niehrs, 2016; Taelman et al., 2010). Wnt/STOP is suggested to play a role in, amongst others, cell size control (Acebron et al., 2014), cell division (Huang et al., 2015) and chromosome segregation (Lin et al., 2021).

1.4. Other β-catenin independent Wnt/signaling pathways

Besides Wnt/STOP signaling, other β-catenin independent Wnt signaling pathways can regulate cellular behavior. GSK-3β, which is best known for its role in marking proteins for proteasomal degradation (e.g. β-catenin), can also modulate the activity of target proteins by phosphorylation. For example, Wnt/LRP6 signaling activates the target of rapamycin (TOR) pathway through inhibition of GSK-3β. The TOR pathway is an important regulator of, amongst other processes, cellular metabolism and protein synthesis (Panwar et al., 2023). When no Wnt ligands are present, GSK-3β can phosphorylate tuberous sclerosis complex 2 (TSC2). Phosphorylation of TSC2 promotes the binding of this complex to TOR complex 1 (TORC1), leading to the inhibition of TORC1. In presence of Wnt, GSK-3β can no longer phosphorylate TSC2, resulting in the activation of the TOR pathway and subsequent increase in protein synthesis (Inoki et al., 2006).

2. The bidirectional relationship between the cell cycle and Wnt signaling

The cell cycle is essential for reproduction, development, growth, and maintenance of organisms. It is becoming increasingly clearer that Wnt signaling is not only an important regulator of the cell cycle, but that the cell cycle also controls Wnt signaling (Habib & Acebrón, 2022; Niehrs & Acebron, 2012). Indeed, it has been shown that Wnt signaling can promote cell proliferation trough regulating cell cycle checkpoints and effectors such as cyclins and cyclin-dependent kinases (CDKs). On the contrary, the efficiency of Wnt signal transduction is also regulated by the cell cycle.

Box 1: Brief overview of the cell cycle

The cell cycle can be classified into distinct phases, the interphase and the mitotic phase. The interphase consists of a first gap period (G1), a DNA synthesis phase (S phase), and a second gap period (G2). During the mitotic phase, the cell undergoes chromosome segregation (mitosis) and cell division (cytokinesis). The progression from one phase to the next is controlled by checkpoints, which are regulated by cyclin-dependent kinases (CDKs). However, CDKs are only active when they are bound to their specific cyclin (Barnum & O'connell, 2014; L. Liu et al., 2019)

In **G1**, the cell is actively growing by increasing protein synthesis while carrying out its normal functions. The length of G1 can vary greatly, depending on the conditions of the extracellular environment. If the conditions of the cell's environment are unfavorable, the cell can exit the cell cycle into a ''resting phase'' called **G0**. When the cell's environment is favorable, the cell will pass the G1/S checkpoint, called the restriction point, and commit to DNA replication and cell cycle entry (Bruce Alberts et al., 2015). This transition from G1 to S phase is regulated by cyclin D/CDK4/6-complex (Baldin et al., 1993), and cyclin E with the associated CDK2 (Dulić et al., 1992). Together, these complexes phosphorylate and inactivate the retinoblastoma protein (Rb) (Hinds et al., 1992), leading to the release of E2F transcription factors, which are required for the activation of genes necessary for DNA replication .

During **S Phase,** chromosome duplication takes place to prepare for mitosis. Helicases unwind the DNA so that DNA polymerases can duplicate the DNA, creating a pair of chromosomes that are tightly held together. S phase progression is controlled by cyclin A and CDK1/2, which get upregulated during this phase of the cell cycle (Stead et al., 2002).

After DNA synthesis, **G2** follows. During this phase, the cell continues to grow and duplicates organelles to prepare for mitosis. At the end of G2, another checkpoint regulates whether cells can enter mitosis. Important factors are a favorable environment and correctly duplicated DNA. The transition from G2 to the next phase is regulated by the translocation of cyclin B to the nucleus, where it forms an activated complex with CDK1 to drive chromosome segregation (Gavet & Pines, 2010; L. Liu et al., 2019).

After passing the G2/M checkpoint, the cell enters the **M phase**, in which the chromosomes are segregated (mitosis) and the cell is divided into two daughter cells (cytokinesis) (Bruce Alberts et al., 2015) (Box 3).

2.1. Wnt in cell size control

During interphase, as cells prepare to divide, they are actively growing by increasing protein synthesis and organelle duplication. This process ensures that each daughter cell obtains a sufficient set of organelles and other cellular components necessary for survival and functioning after mitosis (Jamasbi et al., 2022). Interestingly, accumulating evidence reveals that Wnt signaling is an important regulator of cell size. One mechanism through which β-catenin independent Wnt signaling controls cell size is through TOR pathway activation in G1 (Inoki et al., 2006). The TOR pathway regulates cellular metabolism and protein synthesis (Panwar et al., 2023), which is important during cell growth. Inoki and colleagues have shown that activation of the Wnt pathway leads to the inhibition of TSC2, a TOR pathway inhibitor, thus subsequently leads to TOR activation. They showed that this TOR pathway activation was dependent on the activity of 5' AMP-activated protein kinase (AMPK). AMPK, which is important for cellular energy homeostasis, provides a priming phosphorylation site on TSC2. In the absence of Wnt signals, TSC2 is recognized by the primed phosphorylation site and phosphorylated by

GSK-3β, which inhibits the TOR pathway. Upon Wnt activation, GSK-3β can no longer phosphorylate TSC2 and thus activates the TOR pathway. In line with these results, using co-immunoprecipitation, Mak et al., 2003 have shown that TSC2 can indeed interact with GSK-3β, Dvl, and Axin. Interestingly, inhibition of the TOR pathway using rapamycin only partially inhibited the Wnt effect on cell size, indicating a second mode of action might play an additional role (Inoki et al., 2006). A more recent study has shown that Wnt/STOP can also regulate cell size (Acebron et al., 2014), which may play, besides TOR activation, a role in cell size control. In this study, they show that a significant portion of the cytoplasmic protein pool is protected from GSK-3β induced proteasomal degradation through Wnt/STOP signaling. This results in higher levels of proteins and subsequent increase in cell size in G2/M phase in not only transformed cell lines such as HeLa and HEK293T cells, but also in mouse Embryonic Fibroblasts. Interestingly, after blocking the TOR pathway, cell size and protein content still increases upon Wnt activation in G2, suggesting that cell size control in G2 relies on Wnt/STOP and is TOR independent. Thus, protein content in G2 is regulated through Wnt dependent stabilization of proteins, resulting in increased cell size.

2.2. Wnt signaling plays a role in G1 progression

One of the key mechanisms through which Wnt signaling regulates cell proliferation, is through activation of *c-myc* transcription. Upon activation of the canonical Wnt signaling pathway, β-catenin translocates to the nucleus where it binds to TCF/LEF transcription factor. One of the target genes of Wnt/β-catenin signaling is *c-myc*, which has been shown to peak during G1 (Cadigan & Waterman, 2012; Hadjihannas et al., 2012). *c-myc* encodes for a transcription factor which leads to the upregulation of cyclin D (Daksis et al., 1994; García-Gutiérrez et al., 2019), thereby regulating G1 progression. Moreover, c-myc represses p21 through inhibition of transcription (Gartel et al., 2001) and p27 through both inhibition of transcription and induction of p27 degradation (García-Gutiérrez et al., 2019; W. Yang et al., 2001). p21 and p27 both inhibit the cyclin E/CDK2 complex. Thus, upon Wnt activation, p21 and p27 are inhibited, resulting in G1 progression. Moreover, studies have shown that cyclin D and cyclin E are direct targets of GSK-3β, thus are protected from proteasomal degradation upon Wnt activation (Welcker et al., 2003, 2004). Similarly, c-myc is also a direct target of GSK-3β and protected from proteasomal degradation via the Wnt/STOP pathway (Acebron et al., 2014). Thus, protection of c-myc, cyclin D, and cyclin E through the Wnt/STOP pathway could provide another mechanism for Wnt induced control of G1 cyclin levels, and subsequent G1 progression (Figure 3).

2.3. Cell cycle control of Wnt receptor activation

Wnt signaling has been shown to oscillate with the cell cycle, and peak at G2/M (Olmeda et al., 2003), which could be regulated through Wnt receptor activation in G2/M, rather than for example ligand availability. Successful signal transmission after binding of Wnt to Fzd and co-receptor LRP6 relies highly on the phosphorylation of the intracellular domains of LRP6. Upon Wnt binding to the FZD/LRP6 receptor complex, Dvl polymers are recruited, resulting in the formation of a receptor complex signalosome which promotes the phosphorylation of LRP6. LRP6 can be phosphorylated in both a Wnt dependent fashion through GSK-3β, and independently of Wnt (Bilić et al., 2007; Niehrs & Acebron, 2012). Interestingly, the LRP6 receptor can be phosphorylated by CDK14, which is regulated by cyclin Y (Davidson et al., 2009). Cyclin Y levels peak in G2/M phase (Liu et al., 2010), resulting in maximum phosphorylation of LRP6 during G2/M and subsequently enhanced Wnt signaling in G2/M in *C. elegans*,

Xenopus embryos and mammalian cells (Davidson et al., 2009). These studies confirm that the peak of Wnt in G2/M phase is indeed regulated by Wnt receptor activation. The scaffolding protein B-cell CLL/lymphoma 9 (BCL9) prevents the clathrin dependent turnover of the Wnt/LRP6 receptor complex. BCL9 specifically stabilizes the LRP6 signalosome during G2/M, as it is recruited to the receptor complex by the G2/M cyclin B/CDK1-complex, thereby regulating Wnt receptor availability, specifically during G2/M phase (Chen et al., 2018). In line with these results, BioID proximity labeling has shown that BCL9 is a component of the Wnt signalosome (Van Tienen et al., 2017). Thus, together these mechanisms regulate the capability of Wnt receptors to respond to incoming signals, and that this capability of Wnt receptors is highest in G2/M phase. This could explain why Wnt/STOP signaling peaks in G2/M (Acebron et al., 2014). Indeed, a study in the mouse neocortex has shown that cyclin Y is a key regulator of Wnt/STOP signaling (Figure 3) (Da Silva & Niehrs, 2023).

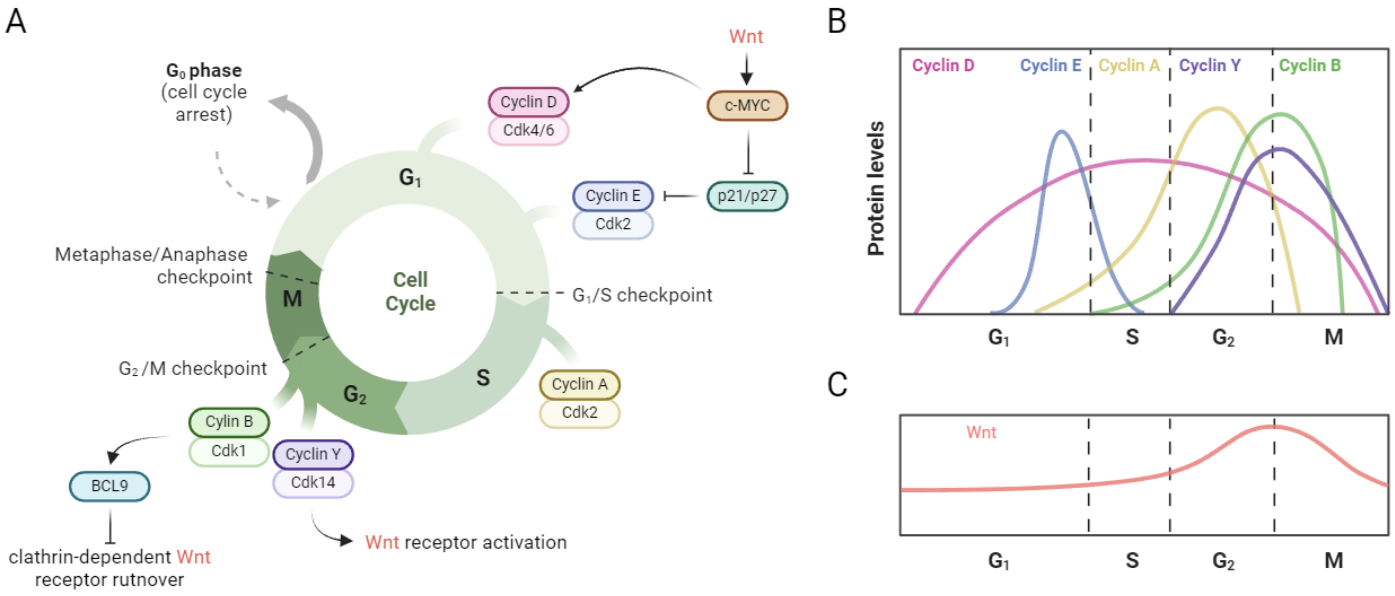


Figure 3: The bi-directional relationship between Wnt signaling and the cell cycle
(A) *During the cell cycle, Wnt directly controls G1 progression trough cyclin D activation by the ß-catenin target gene c-myc. c-myc inhibits p21/p27, which results in increased levels of cyclin E. During G2, the cell cycle controls Wnt receptor availability by inhibiting clathrin dependent Wnt receptor turnover. Moreover, during G2, cyclin Y activates the Wnt receptor, thereby increasing Wnt signaling in G2/M.* ***(B)*** *Levels of the different cyclins during the cell cycle.* ***(C)*** *The levels of Wnt oscillate with the cell cycle. Figure created with Biorender*

3. Wnt signaling in mitosis

Mitosis is the process by which one cell divides into two daughter cells (Box 2). Dysregulation of mitotic processes, e.g. chromosome mis-segregation, can lead to developmental defects and diseases such as cancer (Jamasbi et al., 2022). Therefore, the tight regulation of mitosis is crucial. Besides regulation of G1 and G2 by Wnt, it is becoming increasingly clearer that Wnt signaling is an important regulator of mitosis. Microtubules are a major regulator of mitosis (Bruce Alberts et al., 2015). Components of the Wnt signaling pathway have been shown to be able to directly interact with microtubules, and can therefore regulate aspects of the mitotic spindle. Moreover, Wnt signaling is suggested to play an important role in asymmetric cell division, abscission and the centrosomal cycle. In this part we will

discuss in detail the roles of Wnt signaling pathway components in regulating these essential processes of mitosis.

Regulation of the microtubule cytoskeleton plays a crucial role in the generation of the mitotic spindle, and subsequently in correct cell division. Interestingly, components of the Wnt signaling pathway have been identified to associate with microtubules, and regulate microtubule dynamics. For example, Dvl has been shown to directly bind to microtubules and can increase microtubule stability (Krylova et al., 2000). Moreover, Axin, which is also a component of the destruction complex, can associate with γ-tubulin. γ-tubulin is the main component of the microtubule organizing centers, which are the centrosomes in dividing cells. While the interaction of Axin with γ-tubulin has been shown to regulate microtubule nucleation, the role of Wnt signaling in this process remains to be elucidated (Ruan et al., 2012). Moreover, β-catenin plays a role in microtubule nucleation from the centrosome, as demonstrated by decreased microtubule regrowth after depolarization of the microtubule network upon β-catenin inhibition. Knockdown of Axin-2, a paralog of Axin and a downstream target of Wnt signaling, results in decreased centrosomal nucleation (Huang et al., 2007). Together, this demonstrates the role of Wnt signaling components in the regulation of microtubule dynamics.

3.1. Wnt directly controls the mitotic spindle

The loss of cohesion of the two centrosomes is crucial for the formation of the bipolar mitotic spindle. Interestingly, β-catenin has been shown to contribute to centrosome cohesion (Bahmanyar et al., 2008). Axin-2 is upregulated during mitosis (Davidson et al., 2009). Additionally, Axin-2 localizes to the centrosomes in mitotic cancer cells (Hadjihannas et al., 2006), which is mediated by the interaction with polo-like kinase 1 (PLK1). Mechanistically, the centrosomal pool of Axin-2 induces β-catenin phosphorylation through GSK-3β, which is important for centrosomal cohesion (Hadjihannas et al., 2010). Activation of the Wnt pathway blocks the phosphorylation of β-catenin, and thereby promotes centrosomal splitting (Hadjihannas et al., 2010), highlighting the role of Wnt signaling components in centrosomal cycle.

At the start of mitosis, the microtubule network needs to be drastically remodeled to form the mitotic spindle. Interestingly, LRP6-dependent Wnt signaling has been shown to recruit the Kinesin family member 2A (KIF2A), a minus-end microtubule depolymerase, to the mitotic spindle. This recruitment is dependent on Dvl, which interacts with the motor-domain of KIF2A, and PLK1. PLK1 localizes to the centrosomes and kinetochores (Schmucker & Sumara, 2014) and its interaction with Dvl and KIF2A is crucial for the recruitment of KIF2A to the mitotic spindle. During prometaphase, KIF2A activity within the spindle generates pulling forces on kinetochores, thereby regulating chromosome alignment. As a consequence, as demonstrated by both Wnt inhibition and KIF2A knockdown, mislocalization of KIF2A results in chromosomal misalignment (Bufe et al., 2021). Moreover, BCL9, which stabilizes the LRP6 signalosome during G2/M phase, localizes to the spindle poles. Knockdown of BCL9 resulted in aberrant spindle orientation and misaligned chromosomes (Figure 4A).

Box 2 : Brief overview of Mitosis in animal cells

Mitosis is the process by which chromosomes are segregates, and a single cell divides into two genetically identical daughter cells. Before mitosis, the centrosome has duplicated, and these two organizers of the microtubule network and mitotic spindle remain closely localized to the nucleus.

The first phase during mitosis is the **prophase** in which the duplicated sister chromatids, which are still closely held together, condense. The two centrosomes move apart and from a mitotic spindle through the nucleation of new microtubules.

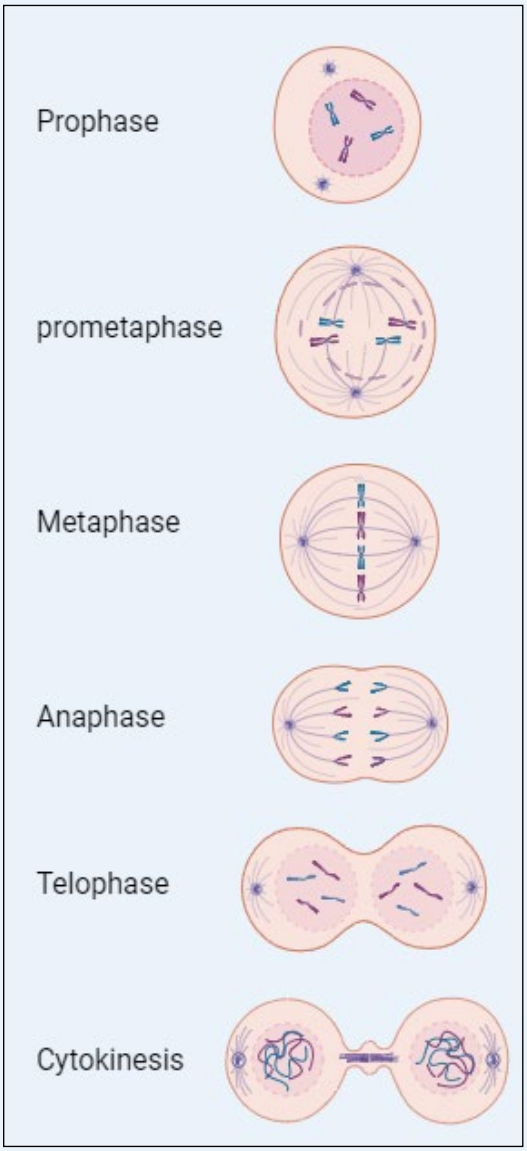
Next, the **prometaphase** starts with the abrupt and fast breakdown of the nuclear envelope. The chromosomes can now attach to the microtubules of the mitotic spindle via their kinetochores, which will induce active movement of the chromosomes.

During **metaphase,** the chromosomes are aligned at the equator of the mitotic spindle, or the metaphase plate. Before progressing to the next phase of mitosis, cells must pass through the metaphase/anaphase checkpoint. This checkpoint prevents the segregation of chromosomes before all chromosomes are properly aligned at the metaphase plate.

In **Anaphase** the Anaphase Promoting Complex or Cyclosome (APC/C) promotes the loss of cohesion between the two sister chromatids (Barnum & O'connell, 2014). Subsequently, the sister chromatids are synchronously pulled to the spindle poles they are facing, facilitated by the shortening of the kinetochore microtubules.

In **telophase**, the two sister chromatids arrive at the spindle poles and decondense. Next a new nuclear envelope is formed around each set of chromosomes, and the spindle poles are dissembled.

Cytokinesis is the process through which the cell is divided into two daughter cells. It involves the formation of an actomyosin contractile ring at the cleavage site, which pinches and separates the cytoplasm into two. During **abscission**, which is the last stage of cell division, the membrane between the two daughter cells is cut (Kodba & Chaigne, 2023). This process marks the completion of cell division, resulting in two genetically identical daughter cells.



Text adapted from: Alberts et al, 6th edition
Figure created with Biorender

3.2. Wnt signaling in chromosome segregation

Mutations in the Wnt signaling pathway are often associated with cancer, as Wnt signaling has been shown to be an essential regulator of chromosome segregation. Indeed, the inhibition of Wnt signaling, though genetic perturbation resulting in the loss of Wnt secretion, results in whole chromosome mis-segregation in mouse embryonic stem cells (Augustin et al., 2017). Stolz et al., 2015 have shown that loss of LRP5/6 or Dvl causes abnormally fast growth rate of microtubules in human somatic cells. This increase in microtubule growth rate is dependent on Wnt/STOP and resulted in failed attachment of the mitotic spindle and subsequent mis-segregation of chromosomes. However, the exact mechanism through which Wnt/STOP regulates microtubule growth rate is not yet understood (Stolz et al., 2015).

Following up on this study, Lin et al., 2021 propose that the regulation of faithful mitosis relies on autocrine Wnt10b signaling, as inhibition of Wnt secretion resulted in chromosome mis-segregation. In line with these results, inhibition of GSK-3β using small molecule inhibitors can similarly lead to chromosomal instability, suggesting this pathway is indeed Wnt/STOP dependent. APC, a component of the destruction complex, localizes to the centrosomes. Moreover, APC interacts with the dynamic ends of microtubules, which is required for connecting them to kinetochores (Fodde et al., 2001). Mutation in APC are often associated with cancer and chromosomal instability. Indeed, these mutations often impair the linkage between microtubules and kinetochores resulting in chromosome mis-segregation (Hadjihannas et al., 2006). These different studies highlight the essential role of Wnt in chromosome segregation (Figure 4B).

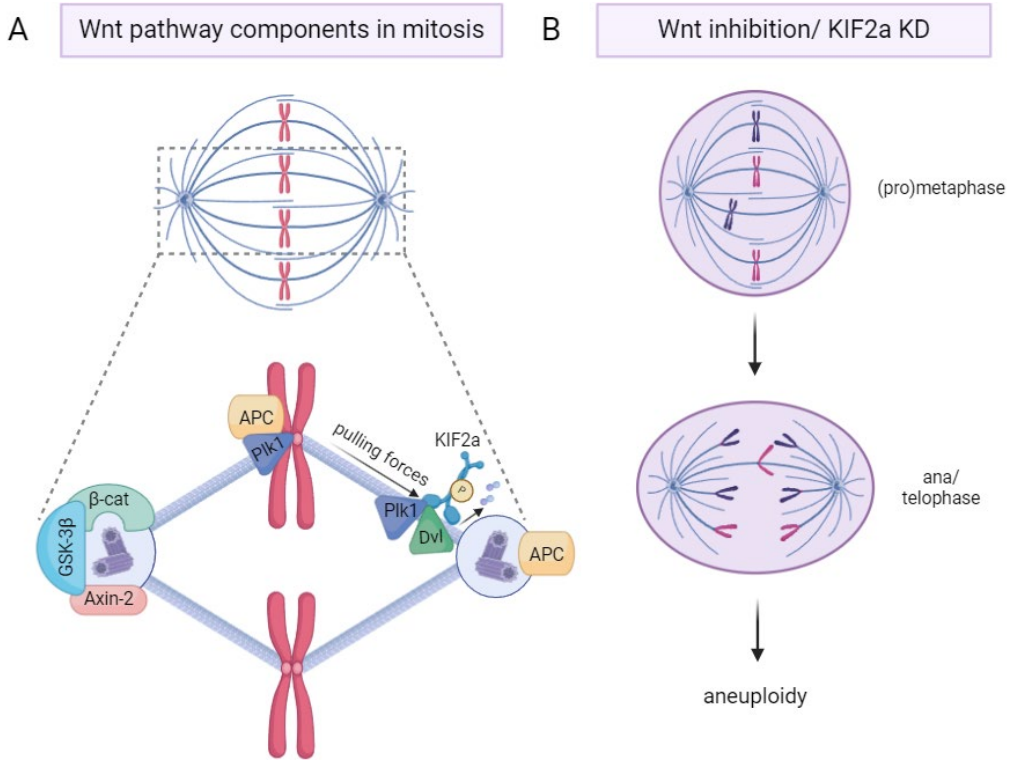


Figure 4: Wnt signaling components regulate mitosis and chromosome segregation

(A) *Wnt signaling pathway components interact with the centrosomes, kinetochores and mitotic spindle. The centrosomal pool of Axin-2 induces the phosphorylation of β-catenin through GSK-3β. APC localizes to the centrosomes and interacts with the dynamic ends of microtubules, which is important for connecting them to kinetochores. PLK1 localizes to the kinetochores and centrosomes and recruits KIF2A to the mitotic spindle together with Dvl.* ***(B)*** *Wnt inhibition and KIF2A knockdown results in chromosomal instability, and subsequent chromosome mis-segregation. Figure created with Biorender*

3.3. Wnt signaling controls asymmetric cell division

Cell division can result in the production of either two identical daughter cells (symmetric division) or two different daughter cells (asymmetric division). During development, the division of cells is often asymmetrical, giving rise to daughter cells with distinct fates. Here we discuss how asymmetric cell division (ACD) is controlled by Wnt signaling in different organisms.

C. elegans:

During the development of the *C. elegans* embryo, a distinct pathway, the Wnt/β-catenin asymmetry pathway (WbA), gets activated during multiple asymmetric cell divisions. Here, in the early embryo (4 cell stage), a precursor cell secretes the *C. elegans* homolog of Wnt, MOM-2, to one side of the neighboring cell. In turn, this cell orients the mitotic spindle towards the Wnt source, resulting in an

ACD (Lam & Phillips, 2017). The direction of the mitotic spindle is regulated by a cortical actomyosin flow, generated upon cell-cell contact, and when a Wnt signal is present (Sugioka, 2018). After ACD, the Wnt-proximal cell will inherit low levels of the destruction complex, which in turn will activate the translocation of the β-catenin homolog Sys-1 to the nucleus to regulate gene transcription (X. D. Yang et al., 2011).

Mammals:

ACD cell division of mammalian cells is predominantly regulated by the Wnt/PCP pathway, which in turn regulates the mitotic spindle orientation. Interestingly, ACD of the mouse neocortex is regulated by the Wnt/STOP pathway (Da Silva et al., 2021). The Wnt/STOP pathway stabilizes the levels of SOX4 and SOX9 proteins, which are important for neuronal differentiation. Moreover, Wnt/STOP signaling has been shown to be an important regulator of the spindle positioning. Indeed, neurogenesis in mice mutant for cyclin Y, a key regulator of the Wnt/STOP pathway, was impacted through reduced ACD caused by improper spindle positioning (Da Silva et al., 2021). In mouse embryonic stem cells, Wnt signals are high on one side of the cell, which is important for spindle positioning. Wnt receptors, β-catenin and APC localize to one side of the cell and position the older mother centrosome towards the Wnt gradient, therefore positioning the mitotic spindle perpendicular to the Wnt source (Habib et al., 2013).

3.4. The role of Wnt in cytokinesis

The last event of cell division is abscission of the membrane that connects the two daughter cells. During anaphase, parallel microtubules form a spindle midzone between the membrane, forming the central microtubule spindle (figure 4). As cytokinesis progresses, an actomyosin ring forms around the membrane connecting the two daughter cells, creating a so called cleavage furrow. Through this narrowing of the cleavage furrow, an intracellular bridge forms, containing a structure called the midbody in the center. This midbody is essential for the recruitment of proteins involved in the scission of the membrane. These proteins include the endosomal sorting complex required for transport III (ESCRT-III), which forms filaments around the midbody. Together with associated proteins, ESCRT-III filaments bend around the midbody and subsequently pinch of and seal the membrane bridge that connects the two daughter cells (reviewed elsewhere in: Kodba & Chaigne, 2023).

Two decades ago, Kaplan et al., 2004 already showed that β-catenin, the most important effector of the canonical Wnt signaling pathway, localizes at the midbody in different mammalian cell types. While they did show that mitotic spindle assembly was impaired upon reduction of β-catenin levels, the potential role of β-catenin on the midbody, or during abscission, was not addressed. More recently, a study from Yu et al., 2021 addressed the functional localization of β-catenin at the midbody. Epithelial cell transforming 2 (ECT2) and RhoA are essential regulators of the narrowing of the cleavage furrow (Chalamalasetty et al., 2006; Watanabe et al., 2008). During early telophase, PLK1 phosphorylates β-catenin on a novel Ser60 phosphorylation site, which subsequently results in the recruitment of ECT2 to the central spindle. ECT2 localization to the central spindle is in turn important for narrowing of the cleavage furrow through the actomyosin contractile ring and completion of cytokinesis. Indeed, β-catenin depletion resulted in cytokinesis defective phenotypes and apoptosis through the decreased interaction between PLK1 and ECT2. However, Wnt activation or inhibition did not cause significant changes in midbody localization of β-catenin. This suggests that this role of β-catenin is independent of the canonical Wnt signaling pathway (Yu et al., 2021). During abscission of naïve pluripotent stem cells, sustained Aurora B signaling delays abscission trough the stabilization of microtubules (Kodba et

al., 2024). When cells exit pluripotency, the abscission rate becomes faster (Chaigne et al., 2020). During the exit of pluripotency, a decrease in Wnt signaling leads to a decreased Aurora B activity, thereby increasing the abscission rate (Kodba et al., 2024). This suggest an important role of Wnt signaling in abscission rate and in the control of pluripotency.

Different Wnt ligands can induce different downstream pathways. Wnt5a specifically activates a β-catenin independent pathway via the Fzd/ROR2 receptor complex. Fumoto et al., 2012 have shown that during cytokinesis, Dvl2 localizes to the midbody where it is required to stabilize the parallel microtubules. Interestingly, Fzd similarly localized to the midbody, where it recruits, and forms a complex, with CHMP4B, a subunit of the ESCRT-III complex. Together, this study shows that β-catenin independent Wnt5a signaling is required for midbody architecture and proper positioning of the ESCRT-III complex, highlighting a role for Wnt signaling in abscission (Fumoto et al., 2012).

Concluding remarks

Since the discovery of Wnt signaling almost 40 years ago, a plethora of studies have identified mechanisms of Wnt signaling affecting the cell cycle and mitosis. Here, we aimed to give an overview of the complex interplay between Wnt and the cell cycle, and discuss the roles of Wnt signaling during mitosis. During interphase, Wnt controls G1 phase progression through the activation of *c-myc* transcription. Wnt signaling controls the cell size in both G1 and G2 as cells prepare to divide. In G2/M phase, the cell cycle controls Wnt receptor availability and activity. During mitosis, different components of the Wnt signaling pathway directly control the mitotic spindle. Moreover, Wnt signaling plays a crucial role in asymmetric cell division by controlling the spindle position. Furthermore, Wnt signaling components interact with the central spindle during abscission and Wnt signaling controls abscission rate in pluripotent stem cells.

Fluorescent tagging of Wnt ligands is very hard, since this often interferes with the secretion and functioning of the protein. While tagging Wnt with a small HA tag is possible, the protein cannot be followed live in order to better understand the spatio-temporal regulation of Wnt signaling. Therefore, live imaging of Wnt mainly relies on tagging downstream targets of the Wnt signaling pathway. Thus, studies mainly rely on gain and loss of function studies.

While many Wnt signaling pathway components, such as Axin, Dvl and APC have been shown to play a role in mitotic spindle organization, the contribution of Wnt signaling pathway activation in this context is not fully understood. Moreover, an outstanding question is whether Wnt signaling pathway activation can contribute to the regulation of the localization and levels of these proteins on the mitotic spindle. While it has been shown that for example, β-catenin localization on the midbody is unaltered upon Wnt activation (Yu et al., 2021), midbody architecture can be controlled through Wnt signaling (Fumoto et al., 2012). The crosstalk between these two distinct mechanisms of midbody architecture remains to be elucidated.

Wnt signaling is an important regulator of development and homeostasis. Dysregulation in the Wnt signaling pathway is implicated in many diseases such as cancer, coronary artery disease and late-onset Alzheimer's disease (Parsons et al., 2021; M. Yu et al., 2023). Oncogenic mutations in the Wnt signaling pathway, leading to hyper-proliferation, are one of the leading causes of many different cancers, emphasizing the pathways' importance in cell proliferation (Parsons et al., 2021). Therefore, obtaining detailed insight into the mechanism of how Wnt signaling controls proliferation and chromosome segregation is valuable.

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