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REVIEW ARTICLE

Canonical and noncanonical Wnt signaling: Multilayered mediators, signaling mechanisms and major signaling crosstalk

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KEYWORDS
β-catenin;
Canonical Wnt;
Noncanonical Wnt;
Signal transduction;
Signaling crosstalk

Abstract Wnt signaling plays a major role in regulating cell proliferation and differentiation. The Wnt ligands are a family of 19 secreted glycoproteins that mediate their signaling effects via binding to Frizzled receptors and LRP5/6 coreceptors and transducing the signal either through β-catenin in the canonical pathway or through a series of other proteins in the noncanonical pathway. Many of the individual components of both canonical and noncanonical Wnt signaling have additional functions throughout the body, establishing the complex interplay between Wnt signaling and other signaling pathways. This crosstalk between Wnt signaling and other pathways gives Wnt signaling a vital role in many cellular and organ processes. Dysregulation of this system has been implicated in many diseases affecting a wide array of organ systems, including cancer and embryological defects, and can even cause embryonic lethality. The complexity of this system and its interacting proteins have made Wnt signaling a target for many therapeutic treatments. However, both stimulatory and inhibitory treatments come with potential risks that need to be addressed. This review synthesized much of the current knowledge on the Wnt signaling pathway, beginning with the history of Wnt signaling. It thoroughly described the different variants of Wnt signaling, including canonical, noncanonical Wnt/PCP, and the noncanonical Wnt/Ca^{2+} pathway. Further description involved each of its components and their involvement in other cellular processes. Finally, this review explained the various other pathways and processes that crosstalk with Wnt signaling.
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Introduction

Wnt signaling is an important evolutionarily conserved pathway that regulates a diverse range of cellular activities. The importance of these pathways in cell proliferation, differentiation, and migration has led to extensive study of its various components, but there is still much to be learned and discovered about this pathway and its various interactions with other pathways. The extent of the research on this pathway has led to it becoming the subject of many evolving therapies. Many of its components have been implicated in the development of treatments for various conditions, such as cancer, neurodegenerative diseases, congenital disorders, and even diabetes and heart disease. Wnt signaling is truly a fundamental pathway to much of human development and health.

The discovery of the first Wnt gene dates back 40 years ago to 1982 during experiments intended to discover proto-oncogenes via activation by proviruses.[1,2] These experiments led to the discovery of *int1*, which causes a tumor of mammary epithelial cells when activated.[1] Eventually, it was discovered that mice and human *int1* homologs shared 99% of their amino acid sequences, elucidating the high degree of conservation of the *int1* proto-oncogene.[3] Prior to this, Sharma and Chopra identified a gene that codes for the development of the wings in *Drosophila melanogaster*, naming the gene wingless (*Wg*).[4] Additionally, molecular hybridization identified a similar *int1* homologue in *Drosophila*.[5] This gene was initially called *Dint1*, but isolation and sequencing of clones of the *Dint1* gene determined that it was identical to the *Wg* gene.[5–7] Later, it was decided that the *int1* and *Wg* genes would be named together as *Wnt1* to reduce confusion with the other int genes. *Int2* was renamed to *FGF3*, *int3* was renamed to *Notch4*, and *int4* is now called *Wnt3A*.[8] The Wnt ligands are now known to be a group composed of 19 glycoproteins that each bind receptors at the cell surface to trigger intracellular signaling cascades to modulate gene expression.[9–13] Each of the Wnt ligands is a cysteine-rich protein that is 350–400 amino acids in length, with an N-terminal signal sequence targeting them for secretion (Table 1).[14]

Wnt ligand family: canonical *vs*. noncanonical

Canonical and noncanonical Wnt ligands

Wnt signaling can be categorized into two pathways, the β-catenin-dependent pathway (canonical) and the β-catenin-independent pathway (noncanonical).[15,16] The canonical pathway is important for inducing cell proliferation, differentiation, and maturation.[16] It is also vital in producing proper body-axis specifications.[17] The pathway is activated via the Wnt1 class ligands, which include Wnt2, Wnt3, Wnt3a, and Wnt8a.[17] The canonical pathway is associated with the transport of β-catenin to the nucleus upon Wnt binding to the Frizzled (Fz or Fzd) receptor and the coreceptors LDL-receptor-related proteins 5 and 6 (LRP5 and LRP6)[18,19] (Fig. 1). The Fz receptor contains a cysteine-rich domain (CRD) that is used to bind Wnt.[20] In mammals, 10 Fz receptors have been identified.[17,21]

If Wnt ligand binding does not occur, a destruction complex that is normally inhibited by Wnt removes the β-catenin. This destruction complex is composed of adenomatous polyposis coli (APC) protein, Axin, serine/threonine kinase glycogen synthase kinase 3 (GSK-3), casein kinase 1 (CK1), the E3-ubiquitin ligase β-TrCP, and protein phosphatase 2A (PP2A).[22] While PP2A can impact Wnt signaling

Table 1 List of Wnt genes and orthologs in humans and model organisms.

Human		Mouse		Xenopus	Zebrafish	Drosophila
Wnt Gene	Chr	Ortholog	Chr	Ortholog	Ortholog	Ortholog
WNT1	12	*Wnt1*	15	*Wnt1*	*Wnt1*	*Wg*
WNT2	7	*Wnt2*	6	*Wnt2a*	*Wnt2*	
WNT2B/13	1	*Wnt2b/13*	3	*Wnt2b*	*Wnt2b*	
WNT3	17	*Wnt3*	11	*Wnt3*	*Wnt3*	
WNT3A	1	*Wnt3a*	11	*Wnt3a*		
WNT4	1	*Wnt4*	4	*Wnt4*	*Wnt4a & Wnt4b*	
WNT5A	3	*Wnt5a*	14	*Wnt5a*		
WNT5B	12	*Wnt5b*	6	*Wnt5b*	*Wnt5b*	
WNT6	2	*Wnt6*	1	*Wnt6*		*DWnt6*
WNT7A	3	*Wnt7a*	6	*Wnt7a*	*Wnt7 & Wnt7a*	*DWnt2*
WNT7B	22	*Wnt7b*	*15*	*Wnt7b*		
WNT8A	5	*Wnt8a*	18	*Wnt8a*	*Wnt8a*	*DWnt8/WntD*
WNT8B	10	*Wnt8b*	19	*Wnt8b*	*Wnt8b*	
WNT9A	1	*Wnt9a/14*	11			*DWnt4*
WNT9B		*Wnt9b/15*	11			
WNT10A	2	*Wnt10a*	1	*Wnt10a*	*Wnt10a*	*DWnt10*
WNT10B/12	12	*Wnt10b*	15	*Wnt10b*	*Wnt10b*	
WNT11	11	*Wnt11*	7	*Wnt11 & Wnt11R*	*Wnt11*	
WNT14	1					
WNT16	7	*Wnt16*	6		*Wnt16*	

either positively or negatively in a cellular context-specific fashion,[23] CK1 phosphorylates β-catenin at the Ser45 residue first, in a process called "priming". This enables GSK-3 to phosphorylate the Ser33, Ser37, and Thr41 residues, which ultimately creates the binding site for the β-TrCP protein.[22,24] The β-TrCP protein functions as an adaptor protein that complexes with Skp1/Cullin machinery to ubiquitinate β-catenin, enabling the destruction of β-catenin by the proteasome.[22,24,25]

The binding of Wnt to the Fz receptors and LRP 5/6 transports disheveled protein (Dvl) to the cell membrane, leading to phosphorylation of the cytoplasmic tails of LRP 5/6. The LRP 5/6 can then bind Axin, removing it from the destruction complex, thus causing the complex to disassemble and release the β-catenin.[14,26,27] This results in the stabilization and accumulation of β-catenin in the cytoplasm, and then its translocation to the nucleus and binding with the transcription factors TCF/LEF (T-cell factor/Lymphoid enhancer factor) and thus gene expression.[14,26,28] Without β-catenin, the TCF/LEF complex is joined with the transducing-like enhancer protein (TLE/Groucho), which recruits HDACs, leading to transcriptional repression. However, β-catenin binding to TCF/LEF displaces the TLE/Groucho complexes and leads to the recruitment of activators to modify the interacting proteins, such as CBP/p300, Pygo, BCL9, and BRG1[29] (Fig. 1).

In contrast with the canonical pathway, noncanonical Wnt signaling is β-catenin-independent, as previously mentioned, and involves the Wnt5a type ligands, which include Wnt 4, Wnt5a, Wnt5b, Wnt6, Wnt7a, and Wnt11 [15,17]. Additionally, the noncanonical pathway involves different functions in comparison to the canonical pathway, such as dictating cellular polarization and migration.[17,30] Noncanonical Wnt signaling follows two distinct pathways, the Wnt/planar cell polarity (PCP) pathway and the Wnt/calcium (Ca^{2+}) pathway.[17,30]

The Wnt/PCP pathway, like the canonical pathway, predominantly uses Fz receptors to bind Wnt (Fig. 2). These Fz receptors utilize several coreceptors, including protein tyrosine kinase 7 (PTK7),[31] muscle-skeletal receptor tyrosine kinase (MUSK),[32] tyrosine kinase-like orphan receptor (ROR1/ROR2),[33] tyrosine kinase related receptor (RYK),[34] syndecan,[35,36] and glypican.[37,38] However, the Wnt/PCP pathway also uses Celsr1 and Vangl2 receptors, although the ligand-receptor binding interaction is still relatively unknown[39] (Fig. 2). The Fz receptors in this pathway bind Wnt ligands and phosphorylate Dvl, leading to the recruitment of Inversin (Invs).[40] The polarity protein Par6 interacts with Dvl, and Smad ubiquitination regulatory factor (Smurf) is recruited by the phosphorylated Dvl and binds to Par6. Smurf then ubiquitinates Prickle, a protein that normally inhibits Wnt/PCP signaling, targeting it for proteasomal destruction.[41] The breakdown of Prickle enables Dvl to associate with the Dvl-associated activator of morphogenesis (DAAM). This complex can then activate Ras homologue gene-family member A (RhoA), but not Rac1 or Cdc24.[15,39] DAAM also activates Profilin.[42] Rac1 activates JNK, which phosphorylates and activates c-Jun to go to the nucleus and initiate gene expression.[43,44] JNK also activates CapZ-interacting protein (CapZIP) via phosphorylation.[45] RhoA activates RHO-associated coiled-coil-containing protein kinase (ROCK) and diaphanous 1 (DIA1).[46,47] ROCK activates the myosin II regulatory light chain (MRLC).[48,49] CapZIP, MRLC, DIA1, and profilin all contribute to actin

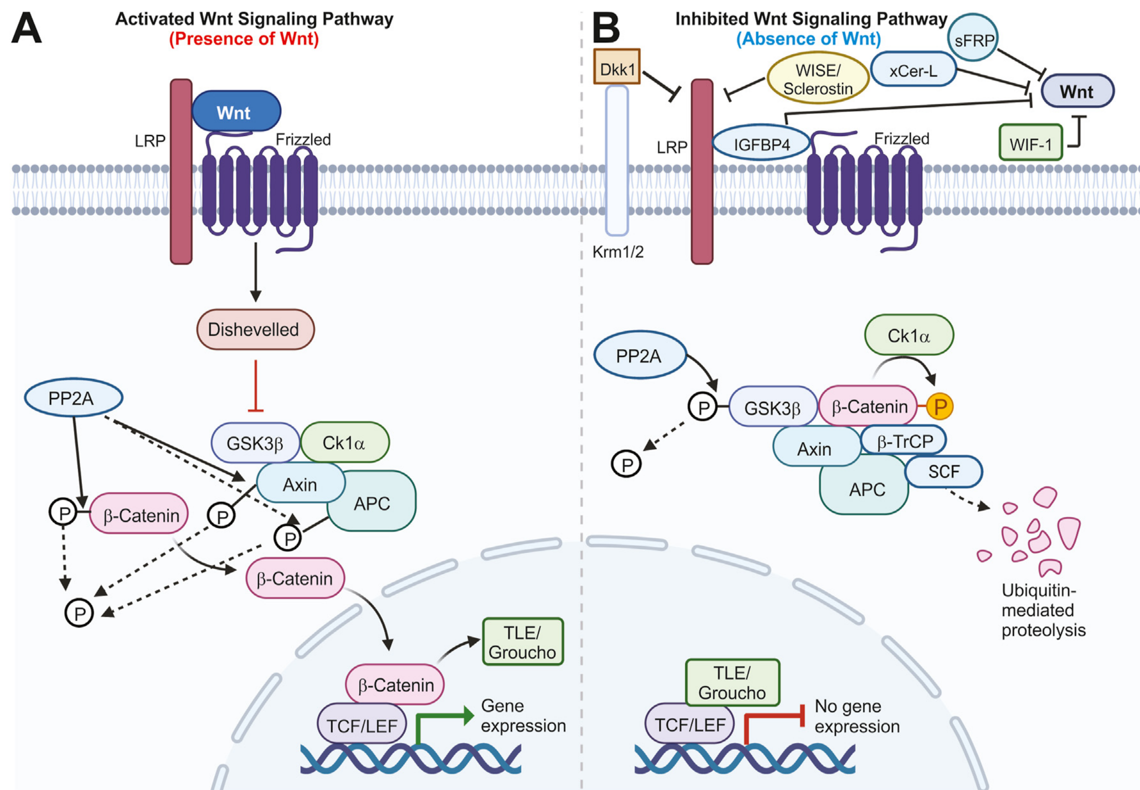

Figure 1 The canonical Wnt signaling pathway. The left panel **(A)** demonstrates the activated Wnt signaling cascade, while the right side portrays the inhibited Wnt signaling cascade. Wnt binds to the Fz receptor and LRP5/6 co-receptor. This activates Dvl to cause the dissociation of Axin from the destruction complex, causing β-catenin to be stabilized and enter the nucleus. β-Catenin can then displace the inhibitory TLE/Groucho complexes, enabling TCF/LEF to transcribe the target genes. PP2A can also enhance Wnt signaling by dephosphorylating β-catenin, APC, and Axin. The result is the preservation of β-catenin by preventing ubiquitination and proteasomal breakdown. In the absence of Wnt signaling **(B)**, the destruction complex breaks down β-catenin and inhibits gene transcription. Several other proteins also contribute to the inhibition of Wnt signaling. Dkk1 associates with Krm1 or Krm2 and LRP5/6, causing endocytosis of the LRP5/6 co-receptor. Wise/sclerostin binds to LRP5/6 to inhibit proper Wnt association with the coreceptor. xCer-L and WIF-1 both bind to Wnt ligands to inhibit signaling. IGFBP-4 functions as a competitive inhibitor of Wnt signaling by associating with LRP6 and Fz8, while sFRPs complex with Fz receptors to prevent Wnt ligand binding. The illustration was inspired by and created in BioRender.

polymerization, which is vital to cell polarity and migration.[39] The Celsr1 receptor appears to function similarly to the Fz receptor, where Wnt binding ultimately causes activation of Dvl to stimulate the same signaling cascade. On the other hand, the binding of Wnt on Vangl2 causes dissociation of a complex of Dvl, Prickle, and inturned (Intu), enabling Dvl to complex with Invs[39] (Fig. 2).

The Wnt/Ca^{2+} pathway is predominantly activated by the Wnt5a ligand and Fzd2 receptor[50,51] (Fig. 3). The binding of the Wnt5a ligand to Fzd2 triggers G protein to activate phospholipase C (PLC).[52,53] PLC then cleaves phosphatidylinositol-4,5-bisphosphate (PtdInsP2 or PIP2) into diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (InsP3 or IP3). DAG, along with Ca^{2+}, activates protein kinase C (PKC) to stimulate cell–division cycle 42 (Cdc42), which causes actin polymerization to contribute to cell polarization and migration.[39] Meanwhile, IP3 binds to inositol-1,4,5-trisphosphate receptors (InsP3Rs) on the membrane of the ER, stimulating Ca^{2+} release through the Ca^{2+} channels and increasing cytoplasmic Ca^{2+} levels.[39,54] Stromal interaction molecule 1/2 (STIM1/2) detects the decrease in Ca^{2+} in the endoplasmic reticulum (ER) and activates Orai family proteins (Orai1, Orai2, or Orai3) on the plasma membrane to mediate store-operated Ca^{2+} entry (SOCE).[39,54] Sarcoplasmic/ER Ca^{2+} ATPases (SERCAs) then pump Ca^{2+} back into the ER.[39,54] The increased cytosolic Ca^{2+} from IP3 binding to InsP3R not only stimulates PKC but also stimulates calcineurin and CAMKII. Calcineurin activates the nuclear factor of activated T cells (NFAT), which causes gene transcription.[55,56] On the other hand, CAMKII stimulates TGF-β-activated protein kinase 1 (TAK1), which then activates Nemo-like kinase (NLK).[57] NLK phosphorylates TCF, inhibiting the β-catenin/TCF complex and preventing gene transcription[58] (Fig. 3).

Biological conservation of Wnt signaling

The Wnt signaling pathway is highly conserved. Wnt was first identified in *Drosophila* as the Wg protein due to mutations in the gene causing lack of wing and haltere development.[4] The Wg gene is also called Dint1, as it was

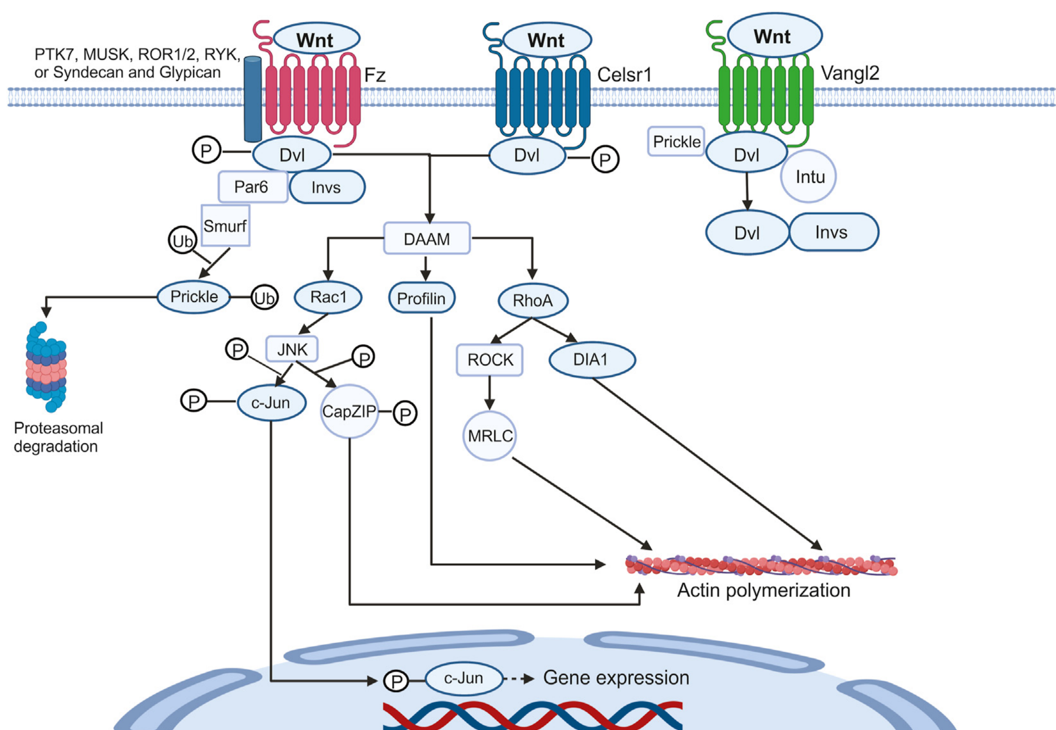

Figure 2 The noncanonical Wnt/PCP pathway. The binding of Wnt ligands leads to the phosphorylation of Dvl, which recruits Invs, Par6, and Smurf. Smurf ubiquitinates the inhibitory protein Prickle, targeting it for destruction. Dvl can then associate with DAAM, activating Rac1, profilin, and RhoA. Rac1 activates JNK, which phosphorylates c-Jun and CapZIP. c-Jun then goes to the nucleus to stimulate gene transcription. RhoA activates ROCK and DIA1, with the latter activating MRLC. CapZIP, MRLC, DIA1, and profilin all stimulate actin polymerization. Celsr1 stimulates Dvl due to Wnt binding like the Fz receptor. Wnt binding to the Vangl2 receptor causes dissociation of Prickle and Intu from Dvl, which can then bind to Invs. The illustration was inspired by and created in BioRender.

shown to be homologous to Int1 found in mice. This was the first connection discovered between Wnt found in *Drosophila* and Wnt found in vertebrates.[7] In an experiment that proved the Wg gene is homologous to Int1 found in mice, van Ooyen et al sequenced the human and mouse Int1 genes in blocks of 50 nucleotides[3] (Table 1). Comparison between the mouse and human Int1 genes revealed conservation of the splice sites, TATA box, and polyadenylation signal.[3] Both proteins were also 370 amino acids long, with the only differences being found in the hydrophobic N-terminus.[3] Homology was also established in the non-coding sequences.[3] The overall homology between mouse and human Int1 homologs was 99%, revealing the conservation of the protein across species.[3]

Studies of cnidarians and sponges have also revealed the conservation of the Wnt signaling pathway, as the pathway was shown to be involved in the control of axis polarity.[59] Petersen and Reddien further clarified that Wnt signaling is broadly used in primary body axis development.[59] The Porcupine gene (Porc) codes for a transmembrane protein in the ER, allowing the processing and distribution of *Drosophila* Wg *in vitro*.[60] Porc is an acyltransferase that palmitoylates the Wnt protein in the ER for secretion.[61] Porc is also important for localizing *Drosophila* Wnt3 in the embryonic CNS.[60] Tanaka et al isolated mouse Porc (Mporc) and Xenopus (Xporc) and analyzed them alongside homologous human MG61, *C. elegans* Mom-1, and *Drosophila* Porc. It was discovered that the Porc homologs among the vertebrates were well conserved via comparison of amino acid sequences, while the *Drosophila* Porc had an additional hydrophilic N-terminal sequence. This study revealed the conservation of Porc across species.[60] Furthermore, injection of Mporc RNA into *Drosophila* embryos with *Drosophila* Porc resulted in some rescue of the embryos, albeit to a reduced extent compared to *Drosophila* Porc RNA. This study revealed the conservation of Porc and Porc function across multiple species.[60]

Biological assays for canonical *vs.* noncanonical Wnt classification

Various biological assays are used to identify components of the canonical and noncanonical pathways. From a broader perspective, the secondary-axis formation (*i.e.*, axis duplication) analysis is one of the prototypes of canonical Wnt assays and can be used to assay activators and inhibitors of the canonical pathway, due to its role in proper axis specification.[62] Luciferase reporter assays revealed that PTK7, a part of the noncanonical pathway, inhibits canonical Wnt signaling by precipitating Wnt3a and Wnt8 in the canonical pathway.[63] Luciferase reporter assays can also be used to detect TCF/LEF activation,[64,65] which are proteins that are mainly involved in the canonical

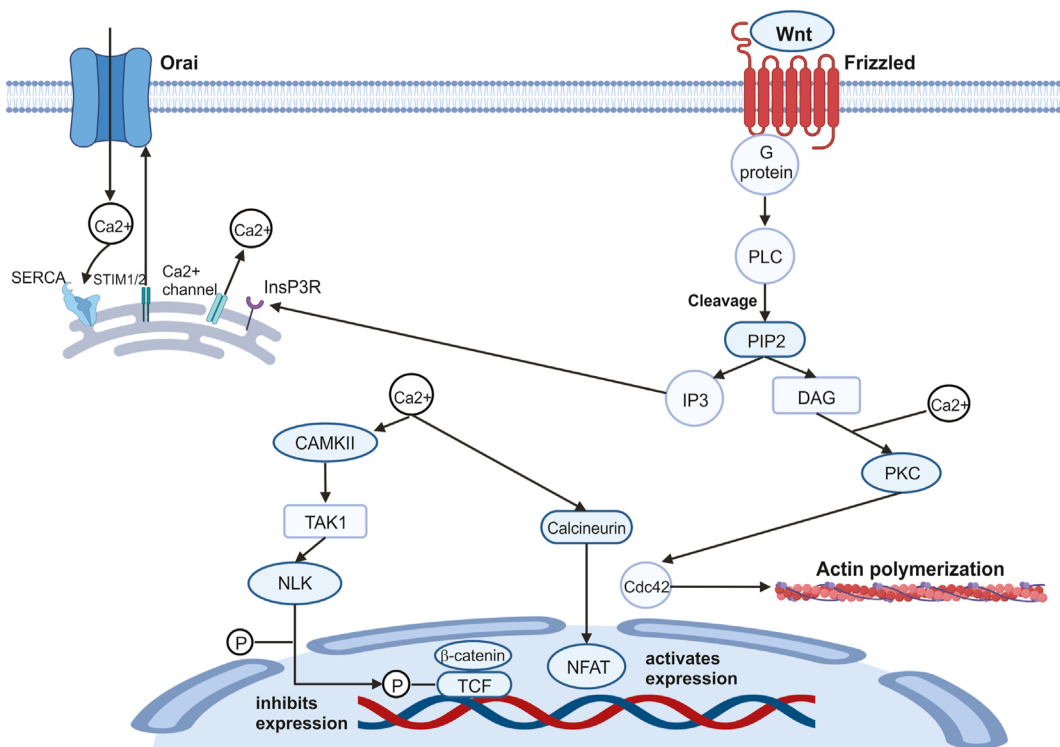

Figure 3 The noncanonical Wnt/Ca^{2+} pathway. Wnt binding to the Fz receptor leads to G protein-mediated activation of PLC. PLC cleaves PIP2 into IP3 and DAG. IP3 binds to IP3 receptors (InsP3R) on the ER membrane to stimulate Ca^{2+} release. STIM1/2 detects this decrease in ER Ca^{2+} levels and activates Orai proteins in the plasma membrane to bring more Ca^{2+} into the cell, where SERCA can pump Ca^{2+} back into the ER. DAG can then activate PKC in the presence of Ca^{2+} and PKC can stimulate Cdc42 to enhance actin polymerization. The elevated intracellular Ca^{2+} level also stimulates calcineurin and CAMKII. Calcineurin activates NFAT, causing gene transcription. CAMKII activates TAK1, which activates NLK, when then phosphorylates TCF, preventing β-catenin-mediated gene transcription. The illustration was inspired by and created in BioRender.

pathway.[28] Luciferase reporter assay could be used to form a high throughput screen for Wnt/β-catenin signaling.[65] Enzyme-linked immunosorbent assay (ELISA) is an assay that can be used to detect Dickkopf-1 (DKK-1), an inhibitor of the canonical pathway.[66] Another type of analysis is the Western blot analysis, which can be used to detect the up-regulation of the canonical proteins β-catenin, Dvl, APC, and GSK-3 in the retina of mice.[66] β-Catenin levels can also be detected using immunodetection assays.[67]

The Wnt inhibitory factor-1 (WIF-1), which is a member of the secreted Frizzled-related protein (sFRP) family, can inhibit both the canonical and noncanonical pathways by binding directly to Wnts, preventing them from binding to the Wnt receptor.[68] Soft agar assay and Western blotting can be used to detect inhibition of osteosarcoma cell growth as a result of WIF-1 overexpression, providing insight into the regulation of both pathways.[64]

In the noncanonical Wnt pathway, Wnt5a is the most prominent ligand, using the ROR family of tyrosine kinases as receptors.[69] A member of the kinesin family, Kif26b, is a downstream effector of the Wnt5a-ROR pathway, mediating cell migration during embryonic development.[70] Hence, a Wnt5a-ROR-Kif26b (WRK) reporter assay was developed and could be used to measure the degree of Wnt5a-ROR signaling in real-time, utilizing a combination of flow cytometry, Western blot, and time-lapse microscopy.[70] A similar test involves a GFP-Kif26b reporter cell line combined with flow cytometry to detect the levels of Wnt5a in the cells.[71] The GFP signal enables quantitative analysis of the cells to detect Wnt5a expression, although the assay is sensitive to cell density.[71]

Controlled secretion of Wnt proteins

Wnt ligands are highly lipidated in the ER, limiting the range of diffusion to localize the ligand to its recipient cell.[72] Specifically, the acyltransferase Porc palmitoylates the Wnt in the ER.[61] The mom-1 gene codes for a similar acyltransferase in *C. elegans*.[73] The Wnt ligands are secreted via a Wntless (Wls) transporter, which is a conserved transmembrane protein in the Golgi apparatus that was characterized in Wg-sending cells in *Drosophila*.[74] Banziger et al transfected embryonic kidney cells (HEK-293T) with the Wnt3a expression vector and cultured them with siRNA to knock down the expression of the human WLS (hWLS) gene. Assaying the level of Wnt3a protein revealed that siRNA-treated hWLS (sihWLS) cells could not activate the Wnt pathway due to, at least in part, the lack of secretion of Wnt3a in the absence of the hWLS gene. This study established the importance of Wls for the secretion of Wnt ligands. Additionally, cell surface heparan sulfate proteoglycans (HSPGs) are also involved in Wnt signaling. Glypicans and syndecans compose the protein core of

HSPGs and are covered in heparan sulfate chains.[75] In *Drosophila*, a glypican called division abnormally delayed (dally) functions as a coreceptor for the *Drosophila* frizzled 2 (Dfz2) receptor.[76] The dally gene specifically encodes the protein core of the HSPGs in Wg/Wnt signaling.[76] Glypicans like dally also participate in transporting Wnt ligands toward target cells.[74]

Wnt receptors, co-receptors, and accessory proteins

Cognate receptors: the frizzled (Fz) proteins

Frizzled (Fz) is a seven-pass transmembrane receptor that binds Wnt ligands.[77] The Fz genes generate Fz receptors, each of which ranges from 500 to 700 amino acids long, with a CRD on the N-terminus and a 40 to 100 amino acid-long hydrophilic linker region.[78] The seven transmembrane domains are hydrophobic alpha-helices.[78] Fz protein localizes in the plasma membrane, with the cysteine-rich N-terminus oriented extracellularly and the carboxy-terminus oriented intracellularly towards the cytoplasm.[21,79,80] The intracellular carboxy-terminus is variable in length and not well conserved between the different Fz receptor types.[78] Fz is also glycosylated[79] and there are 10 Fz genes organized into four clusters.[78] By amino acid sequence, Fzd1, Fzd2, and Fzd7 are 75% similar; Fzd5 and Fzd8 are 70% similar; Fzd4, Fzd9, and Fzd10 are 65% similar; and Fzd3 and Fzd6 are 50% similar.[81] Comparison of amino acid sequences between clusters yields only 20%—40% similarity.[78] The relative lack of sequence similarity suggests a lack of genomic conservation between the Fz receptors.

The importance of Fz receptors in the Wnt/PCP pathway was characterized by Fz mutations in *Drosophila* that caused abnormal wing hair patterns and polarity disruptions, revealing the importance of Fz in the Wnt/PCP pathway.[82,83] Loss of function and overexpression mutations in Fz altered the assembly location of F-actin in wing development, indicating that Fz is important in cytoskeletal development.[79] The misorientation of hairs also revealed the importance of Fz in not only receiving the Wnt signal but also propagating it in a proximal-distal direction.[84] Mutations in Fz receptors also cause the defective orientation of the ommatidia (individual units composing a compound eye).[85]

Mutations in the frizzled 4 (*Fzd4*) receptor causes familial exudative vitreoretinopathy (FEVR), a disease that generally results in the lack of retinal angiogenesis. This leads to a variety of symptoms, including retinal fibrosis, detachment, and dysplasia.[86] Mutations in Fzd1 and Fzd2 in mice caused cleft palate and ventricular septal defects (VSD). These mutations also affected neural tube closure and inner ear development. *Fzd7* mutations also caused VSDs but were more commonly associated with a kinked tail.[87] Knockout of *Fzd5* in mice led to embryonic lethality due to placental insufficiency.[88] Loss of *Fzd5* also caused retinal cell death and excessive mesenchymal cells in the vitreous cavity among other issues.[89] Loss of *Fzd8* alone causes no phenotypic change, although the loss of even a single *Fzd8* allele can increase the severity and penetrance of the ocular effects from loss of *Fzd5*.[90] Homozygous loss of *Fzd9* has yielded B-cell developmental abnormalities, defective visuospatial learning, and bone mass reduction compounded by osteoblast dysfunction.[21] Homozygous Fzd6 deletions cause randomized hair follicles, generating waves, whorls, and tufts.[91] Homozygous deletion of *Fzd3* in mice causes inappropriate development of peripheral and central axons, leading to the inability to detect thermal and mechanical stimuli from the feet.[92] Concomitant deletions in *Fzd3* and *Fzd6* cause neural tube closure defects and misorientation of inner ear hair cells, indicating their similar functions.[93]

Co-receptors: LRP5 and LRP6 proteins

Low-density-lipoprotein receptor-related proteins 5/6 (LRP5/6) are single-pass transmembrane proteins that function as coreceptors for Fz receptors.[19,94] The binding of the Wnt ligand triggers the dimerization of Fz and LRP5/6 [94]. This leads to the phosphorylation of the cytoplasmic tail of LRP5/6 at five conserved PPPSP (also called the PPPSPXS) motifs.[95,96] Several protein kinases are involved in this phosphorylation, including GSK-3, which is part of the destruction complex that normally binds β-catenin in the absence of Wnt signaling.[94,97] The PPPSP motifs bind Axin from the destruction complex, inhibiting β-catenin phosphorylation and subsequent ubiquitination that leads to proteasomal breakdown.[95,96,98] LRP5 and LRP6 are homologous and expressed in embryogenesis.[19] However, LRP6 is more important during embryogenesis, as evidenced by mice with homozygous deletion of *Lrp6* demonstrating axial skeleton truncation, neural tube closure defects like spina bifida, as well as midbrain and hindbrain malformations.[99] On the other hand, homozygous deletion of *Lrp5* caused low bone mass due to decreased osteoblast activity, defective capillary cell apoptosis in the eye, defective clearance of chylomicrons, and impaired insulin secretion.[100,101]

Accessory Wnt binding proteins at the cell membrane

R-spondins

Roof plate specific-spondins (R-spondins) are four members of a larger family that contain thrombospondin type 1 repeats (TSR-1).[102] The R prefix was given based on the first R-spondin due to its expression in the boundary of the roof plate and neuroepithelium in the dorsal neural tube.[103] The R-spondins also contain an N-terminal signal peptide, two furin-like (FU1 and FU2) cysteine-rich domains near the N-terminus, and a C-terminal region with many positively charged amino acids.[102,104] The R-spondins can bind to leucine-rich repeat-containing G-protein coupled receptors 4—6 (Lgr4-6).[105] R-spondins bind Lgr4-6 via their FU2 domain and can bind ZNRF3 and RNF43 via their FU1 domain.[106]

ZNRF3 and RNF43

ZNRF3 and RNF43 are E3 ubiquitin ligases that target Wnt receptors for destruction to decrease Wnt signaling responses.[107] ZNRF3 is a member of the ZNRF protein family, a family of E3 ubiquitin ligases that contain a zinc finger and a RING domain.[108] R-spondins can bridge ZNRF3/RNF43 and

LGR4/5/6, inhibiting ZNRF3/RNF43 activity via auto-ubiquitination and membrane clearance.[106,109] In this manner, R-spondins act as Wnt agonists. RNF is a homolog of ZNRF43 that also contains a RING domain for its function as an E3 ubiquitin ligase.[109]

Derailed/Ryk

Derailed/Ryk (related to tyrosine kinase) is a member of the atypical tyrosine kinase family consisting of a Wnt inhibitory factor (WIF) domain extracellularly, an atypical kinase domain intracellularly, and a PDZ binding motif. Derailed is the *Drosophila* homolog, while Ryk is found in mammals.[110] Interestingly, the tyrosine kinase domain is considered atypical due to sequence variations in the normally conserved tyrosine kinase residues. The domain lacks tyrosine kinase activity.[111] The WIF domain of Ryk binds to Wnt1 and Wnt3a to activate TCF for the transcription of target genes.[110] Ryk also forms a ternary complex with Wnt1 and Fz using its extracellular WIF domain, while the intracellular kinase domain binds to Dvl using its PDZ binding motif to activate TCF in response to Wnt3a stimulation.[110] In *Drosophila*, Derailed was found to be important in learning and memory.[112] Derailed/Ryk was also found to be important in axon guidance via interaction with Wnt5.[113] The C. elegans homolog, lin-18, is important in determining vulval cell fate patterning.[114]

Receptor tyrosine kinase-like orphan receptors (RORs)

RORs are members of the receptor tyrosine kinase (RTK) family that are highly conserved and consist of two members, ROR1 and ROR2.[115] ROR1 and ROR2 are single-pass transmembrane receptors with an intracellular tyrosine kinase domain and a proline-rich domain (PRD) that is flanked by two serine–threonine rich domains.[116] The extracellular side of the ROR contains an immunoglobulin (Ig)-like domain, a CRD, and a Kringle domain (KRD). The CRD of ROR1 and ROR2 is like those on Fz receptors.[117] ROR1 and ROR2 play crucial roles in embryonic development. Mice lacking ROR2 expression have shortened limbs and tails, facial abnormalities, and dwarfism among other issues.[118] Mutations in ROR2 also cause Robinow syndrome and Brachydactyly type B in humans.[119–121] Wnt5a binds ROR2, causing heterodimerization of ROR2 with Fz2 using its CRD. RORs can activate the noncanonical Wnt/JNK-PCP pathway and inhibit the canonical β-catenin/TCF pathway.[122] However, the components of the Wnt/ROR pathways are still mostly unknown.[122] ROR1 is expressed in B-lymphocyte precursors and can rarely cause precursor-B acute lymphoblastic leukemia (B-ALL).[123] ROR1 and ROR2 in mice have been known to play an important role in the development of the nervous system.[124]

Gpr124 and Reck

Gpr124 is a G protein-coupled receptor (GPCR) and Reck is a glycosylphosphatidylinositol-anchored glycoprotein.[125] Reck binds to Wnt7, creating the Reck/Wnt7 complex that binds to Gpr124. This complex then joins with the Fz receptor and LRP5/6 coreceptor to stimulate canonical Wnt/β-catenin signaling.[125–127] The Gpr124 and Reck coactivators are vital in the development of the blood–brain barrier (BBB).[128] *Gpr124* knockout mice demonstrated microvascular hemorrhage and lethality in the embryo. Interestingly, *Gpr124* deletion did not affect BBB integrity in adult mice.[128]

Intracellular mediators of Wnt signaling

The intracellular component of the Wnt signaling pathway is composed of several proteins for each pathway. This section will give a brief overview of each component of the canonical and two noncanonical Wnt pathways, looking further at the additional functions of the components outside of Wnt signaling. A description of the functions in Wnt signaling and the signaling cascade will be described in the canonical *vs.* noncanonical Wnt section. The inhibitors will also be discussed later. For the canonical pathway, the binding of Wnt to Fz and LRP5/6 mediates signal transduction to the nucleus via β-catenin (Fig. 1).

Intracellular mediators of the canonical pathway

β-Catenin was discovered to have two functions, one of them being its association with α- and γ-catenin to link Ca^{2+}-dependent cell adhesion molecules (CAMs) to cytoskeletal structures.[129] The term catenin was given to these three molecules due to its linkage of the CAM, E-cadherin, to cytoskeletal structures.[129] The second function of β-catenin is its role in Wnt signaling, which was discovered through the analysis of its *Drosophila* homolog, Armadillo (Arm). Seminal screens for mutations that caused altered segmentation of *Drosophila* embryos revealed the signaling potential of β-catenin.[130] Additionally, mutations in Wg in *Drosophila* caused a corresponding decrease in Arm, leading to altered segment polarity, further delineating the link between Wnt signaling and β-catenin.[131] Further studies would later reveal that β-catenin mediates its effects via the TCF/LEF transcription factors, stimulating the transcription of target genes.[132]

β-Catenin is a 781 amino acid long protein with 12 Arm repeats, which forms a super-helical structure composed of multiple α-helices with a hydrophobic core.[133] The superhelix contains a large, positively charged groove that allows β-catenin to interact with cadherins, TCF, and APC.[133–139] β-Catenin then binds TCF and LEF to mediate gene transcription (Fig. 4). Interestingly, TCF is considered a transcriptional repressor, while LEF is considered a transcriptional activator.[140] TCF/LEF binds to DNA via its HMG box domain, which recognizes a sequence called the Wnt/Wg response element (WRE) on the DNA.[141,142] The HMG domain binds to the WRE in the minor groove of the DNA, causing the DNA to bend.[142] In addition to the HMG domain, TCF/LEF also contains a nuclear localization signal (NLS) that makes nonspecific contacts with the phosphate backbone of the DNA, increasing TCF/LEF affinity for the DNA.[142]

Intracellular mediators of noncanonical Wnt/PCP pathway

Dvl proteins

There are two noncanonical Wnt signaling pathways, the Wnt/PCP pathway, and the Wnt/Ca^{2+} pathway. The Wnt/PCP pathway involves several additional receptors and co-

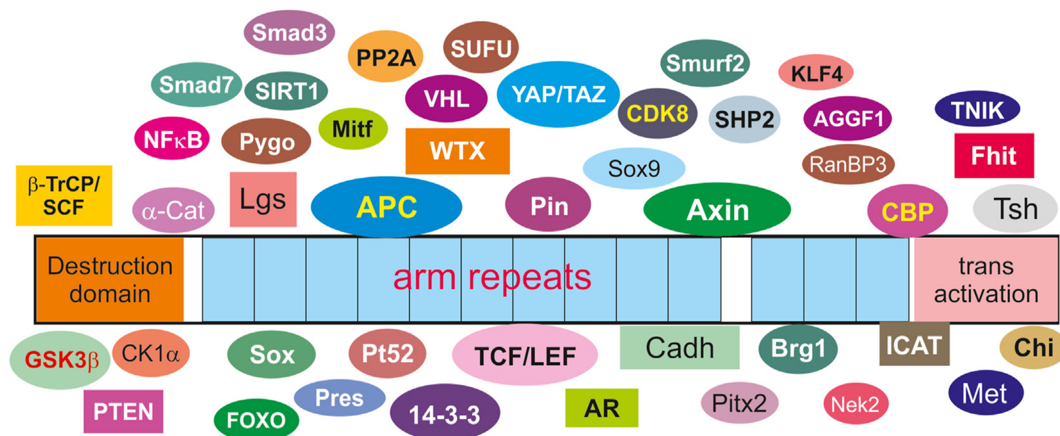

Figure 4 β-Catenin protein interactions. Some of these proteins were not discussed in this paper due to space constraints. Among those that have been discussed in this paper, there are notable inhibitors and activators. GSK-3β, CK1, APC, Axin, and β-TrCP are inhibitors of β-catenin as part of the destruction complex. PP2A has dual effects; it can dephosphorylate β-catenin to prevent ubiquitination and stabilize β-catenin, while it can also dephosphorylate GSK-3β, which can then inhibit β-catenin. YAP/TAZ is another inhibitor, as it can either bind to and suppress β-catenin without affecting its levels or associate with the destruction complex. Smad7 and Smurf2 can complex with β-catenin to ubiquitinate and degrade the protein. SUFU can export β-catenin from the nucleus. On the other hand, there are several activators of β-catenin, proteins that are activated by β-catenin, and proteins that assist with the functions of β-catenin. Smad3 is a chaperone protein that transports β-catenin into the nucleus. TCF/LEF are transcription factors that are activated by β-catenin. α- and γ-catenin join with β-catenin to link CAMs like E-cadherin to cytoskeletal structures, strengthening cell adhesion. It is noteworthy that some of the protein interactions are species-, tissue-, and/or context-dependent. The illustration was inspired by the Wnt homepage created and maintained by the Nusse Lab at Stanford University (http://web.stanford.edu/group/nusselab/cgi-bin/wnt/protein_interactions) and reference [137].

receptors that will be discussed in later sections. The first intracellular mediator of Wnt/PCP signaling is Dvl. All Dvl proteins contain three conserved domains, a DIX domain at the N-terminus, a central PDZ domain, and a DEP domain at the carboxy-terminus.[143] In between the DIX and PDZ domains is a "basic region" composed of conserved Ser and Thr residues. Between the PDZ and DEP domains is a "proline-rich region".[143] The DIX domain primarily activates the canonical pathway, enabling dynamic polymerization of Dvl to form puncta, which can then interact with Axin to prevent it from mediating the destruction of β-catenin.[144] Although further research needs to be conducted to delineate the exact process of this interaction between Dvl and Axin, it has been theorized that the DIX domain of Dvl interacts with the similar DIX domain on Axin to induce a conformational change in Axin or relocates Axin.[144] The PDZ domain is involved in both the canonical and noncanonical pathways.[145] It binds the Fz receptor at its C-terminal conserved Lys-Thr-X-X-X-TRP (KTXXXW) motif.[146] This motif is required for the activation of the canonical pathway, although the molecular mechanisms are poorly understood.[147] The DEP domain activates the noncanonical pathway by mediating the interaction between Dvl and DAAM1.[143] The DEP domain also translocates the Dvl protein to the plasma membrane after Wnt stimulation.[148]

Dvl has an NLS located between the PDZ and DEP domain (aside from the proline-rich region), and an NES (nuclear export signal) located between the DEP and C-terminus.[143] Increasing evidence indicates that nuclear Dvl protein is critical for β-catenin and TCF factors to form a complex.[149] Dvl has been shown to interact with many other transcriptional factors including FOXK1/2, TAZ, and HIPK1, as well as gene promoters such as CYP19A1.[149] Furthermore, Dvl1 was shown to interact with EZH2 while Dvl3 interacts with chromatin-modifying enzymes such as KMT2D in cancer cells. In addition, Dvl proteins are extensively modified post-translationally by phosphorylation, lysine acetylation, and methylation, as well as ubiquitination, although the functional roles of Dvl post-translational modifications remain to be fully investigated.[149]

Inversin (Inv) protein

Inversin (Inv) is a 1062 amino acid long protein with characteristic 15 successive ankyrin repeats. It was first identified in Inv mutant mice that had situs inversus (reversed left/right polarity), underdeveloped tubules, and cyst formation in the kidneys.[150] This discovery linked Inv to Wnt signaling. Simons et al used glutathione S-transferase (GST) fusion protein containing the PDZ domain of Dvl to characterize the effect of Inv. The Inv directly interacted with the GST fusion protein. Inv also formed a protein complex with Dvl, indicating that it could inhibit the canonical pathway.[151] Inv also participates in the noncanonical pathway, interacting with the planar cell polarity (PCP) pathway proteins Strabismus (Stbm) and Prickle (Pk) like the *Drosophila* PCP protein Diego.[151] Inv mutant mice developed renal cysts due to unopposed canonical Wnt signaling, which causes overgrowth of cells without terminal differentiation of renal tubular epithelial cells.[151]

Par6 protein

Par6 serves as a polarity protein and a scaffolding protein with other molecules.[152] The scaffolding function is beneficial in complexing with Dvl and Smurf in the noncanonical

pathway.[41] Its cell polarity function is mediated by the G-protein-activated phospholipase C-Beta (PLC-β) interacting with Par proteins like Par6 through multiple PDZ domains that can bind the extreme C-terminal S/TXL motifs of PLC-β.[152] This activates the PLC-β to hydrolyze PIP2 into IP3 and DAG, two important secondary messengers. IP3 and DAG play important roles in regulating cell polarity and asymmetric cell division.[152]

Smurf1 and Smurf2 proteins

Smurfs (Smurf1 and Smurf2) are E3 ubiquitin ligases of the C2-WW-HECT family of proteins.[153] They were first identified in the ubiquitination and degradation of R-Smads in the BMP pathway to antagonize TGF-β/BMP signaling.[154] Smurf1 contains a phospholipid/Ca^{2+} binding domain on its N-terminus, two WW domains for binding to PPXY (also called PY) motifs on other proteins, and a HECT domain on the C-terminus for ubiquitination of target proteins.[154] Smurf1 targets Smad1 and Smad5 using its WW domains, which bind to the PY motifs on Smad1 and Smad5, enabling their degradation.[154] Smurf2 uses the same mechanism to degrade Smad1 and Smad2.[155] Smurf1 can also be recruited by Par6 to target RhoA for degradation, establishing the proper cell polarity needed for cell movement.[156] Smurf1-induced RhoA degradation in tight junctions leads to their dissolution and enables TGF-β dependent epithelial—mesenchymal transition.[157] These results indicate the importance of Smurfs in noncanonical Wnt/PCP signaling.

Prickle (Pk) protein

Prickle (Pk) is also involved in the Wnt/PCP pathway. Pk is considered a type 1 polarity gene along with Dvl and Fz because it affects the body surface and is believed to directly establish tissue polarity. On the other hand, type 2 and 3 tissue polarity genes affect specific body regions and are believed to interpret the polarity that the type 1 genes have established.[158] All Pk proteins contain three LIM motifs and a conserved domain called Prickle Espinas Testin (PET).[158] The LIM motifs are cysteine-rich domains with two zinc fingers that are joined by an amino acid spacer.[159] The LIM domains bind target proteins and enable protein—protein interactions.[158] The PET domain is monomeric and works with the LIM motifs to target Dvl to the cell membrane to facilitate its function in the Wnt/PCP pathway.[160] Pk also has a prenylation motif on its C-terminus that is important but not required for its localization to the plasma membrane.[161]

Dvl-associated activator of morphogenesis (DAAM)

DAAM is a conserved actin nucleator that is a member of the formin family.[162] DAAM members contain a GTPase binding domain (GBD), a diaphanous inhibitory domain (DID), an N-terminal dimerization domain (DD), a coiled-coil (CC), an FH1 domain, an FH2 domain, and a diaphanous autoregulatory domain (DAD). DAAM is highly expressed in tissues like the CNS, somites, dermomyotomes, and the heart and is important in organ symmetry. Specifically, it causes left-right (LR) symmetry due to its modulation of the myosin 1D (Myo1D) function. *Drosophila* Myo1D induces dextral twisting, which is important for orienting the native LR organs in larvae. The absence of Myo1D leads to situs inversus.[163] DAAM nucleates F-actin, promoting the assembly of an F-actin network that enables Myo1D to induce chirality.[162] Interference of DAAM with RNAi suppressed the expected 180-degree dextral rotation of the larval body, reducing it to just 90°. This experiment revealed the importance of DAAM in inducing proper chirality in *Drosophila* larvae.[162] The FH2 domain is particularly important in actin nucleation and polymerization, while the FH1 domain interacts with profilin-actin during actin filament elongation.[42]

Profilin protein

Profilin is a protein associated with non-muscle actin (β- and γ-actin) and is involved in the control of actin polymerization. It was first isolated from calf spleens as a small protein that accompanied an actin-containing complex.[164] When complexed with actin, profilin is called profilactin.[165] Profilin contains a core of seven-stranded β-pleated sheets with α-helical N- and C-termini on one side, and two shorter α-helices on the other. The N- and C-termini form the poly-L-proline (PLP) binding surface, which enables profilin to bind actin and factors that participate in actin nucleation and elongation.[166] Profilin inhibits spontaneous actin nucleation and polymerization by sequestering G-actin. However, it can also promote actin filament elongation using its PLP binding domain to interact with other proteins such as formins, Ena/vasodilator-stimulated phosphoprotein (VASP), Arp2/3-dependent Wiskott—Aldrich syndrome protein (WASP), and WASP family verprolin-homologous protein (WAVE) family.[166,167] Profilin also facilitates the exchange of ADP for ATP on actin monomers, further enhancing polymerization.[166,167] Interaction with formins allows profilin to associate with microtubules and increases the rate of depolymerization.[168] With profilin as a mediator, DAAM1 can mediate cytoskeletal reorganization and cell movement for important processes such as gastrulation.[169]

Rac1 and RhoA proteins

Rac1 and RhoA are members of the Rho family of small GTPases.[170] Both proteins are involved in a wide variety of cell processes, such as motility, proliferation, migration, and polarity via their regulation of actin polymerization.[171] Both have conserved GDP/GTP binding domains called the G domain and a C-terminal region that contains a CAAX motif.[172] The binding of GTP to Rac1 causes two regions (amino acids 25—40 and 60—76) called switch I and II to undergo a conformational change to interact with specific downstream effectors in the signaling cascade.[171] Rac1 and RhoA can also undergo post-translational modification to prenylate the C-terminal CAAX motif, enabling membrane interaction.[171] This association with the membrane is what enables Rac1 and RhoA to stimulate downstream signaling cascades to modulate cellular functions.[170] The exchange of GDP for GTP is mediated by Guanine Nucleotide Exchange Factors (GEFs) and GTPase activating proteins (GAPs), triggering the active state of Rac1 and RhoA to interact with downstream proteins.[171,173] Guanosine dissociation inhibitors (GDI) can conceal the C-terminal isoprenyl motif in a hydrophobic pocket, sequestering Rac1 in the cytoplasm and preventing downstream activation.[173,174] GDI also prevents GDP/GTP exchange to keep both in their inactive states.[171,174] RhoA and Rac1 are

spatially separated, with Rac1 active towards the leading edge of the cell, while RhoA is active towards the lagging edge.[170] The two are also temporally segregated, with RhoA activity peaking before Rac1 in a coordinated cycle of protrusion and retraction. Rac1 and RhoA can antagonize each other mutually to coordinate this effect.[175] Dysregulation of Rac1 and RhoA have been linked to cancer, as well as cognitive and cardiovascular diseases.[172]

Rho-associated coiled-coil kinase (ROCK)

ROCK (also called RhoA/Rho kinase) is a Ser/Thr kinase that is stimulated by Rho.[46,47,176,177] ROCK comes in two isoforms, ROCK1 and ROCK2. ROCK1 is 1354 amino acids long, while ROCK2 is 1388 amino acids long.[178] They share 64% of their primary amino acid sequences, 92% homology in the kinase domains, and only 55% homology in the coiled-coil domains.[178] The N-terminal region contains the ROCK kinase domains.[178] The C-terminal region contains the coiled-coil domain and a pleckstrin homology (PH) domain; this region binds to the catalytic kinase domain to inhibit its activity. The coiled-coil domain contains a Rho-binding region that enables GTP-bound RhoA to disrupt the binding of the C-terminus and the kinase domain.[179] The C-terminal region can also be cleaved by caspases during apoptosis to activate ROCK.[180,181] Activated ROCK can associate with mammalian DIA (mDia) to stimulate actin cytoskeletal reorganization.[177,182] ROCK proteins are generally expressed in many tissues and phosphorylate target proteins on R/KXXS/T or R/KXS/T amino acid motifs.[178] ROCK proteins are involved in forming stress fibers composed of bundles of F-actin and myosin II.[183] Focal adhesion complexes bind these fibers to the inner plasma membrane.[184] The activation of ROCK by caspases gives ROCK a crucial role in forming membrane blebs during apoptosis.[180,181] ROCK also plays a role in embryonic development, inducing cell migration, differentiation, and axis formation through its expression in the cardiac mesoderm, lateral plate mesoderm, and neural plate.[178]

c-Jun N-terminal kinase (JNK)

JNK is a member of the three mitogen-activated protein kinase (MAPK) pathways that control cell proliferation, migration, and differentiation.[185] The MAPK pathways are all activated by a series of phosphorylation reactions. JNK is activated by the MAP2K enzymes MKK4 and MKK7.[186] Scaffold proteins just as JNK interacting protein 1 (JIP1) facilitate rapid activation of the JNK pathway.[187] The JNK pathway can be inactivated by dual-specificity phosphatases (DUSPs).[188] The JNK family contains three genes that can be spliced into 10 isoforms, namely, JNK1, JNK2, and JNK3. JNK1 and JNK2 are expressed in a variety of tissues, while JNK3 is expressed in the brain, heart, and testis.[185] The protein products of these three genes are about 400 amino acids long, with a canonical Ser/Thr kinase domain.[189] JNK is best known for inducing apoptosis via stimulating the mitochondrial release of cytochrome C to activate caspase and trigger apoptosis.[190] However, the effects of JNK are context-specific, as JNK can phosphorylate anti-apoptotic Bcl-2 to promote apoptosis, but it can also phosphorylate pro-apoptotic BAD protein to prevent apoptosis.[185] The dysregulation of JNK is linked to multiple diseases, such as neurodegenerative disorders, cancer, and autoimmune diseases. JNK3 is being investigated as a potential target for the treatment of CNS disorders.[191]

C-Jun is the major substrate for JNK.[185] c-Jun is a subunit of the transcription factor, activator protein 1 (AP-1).[192] Anti-c-Jun antibodies caused partial G0 arrest in the cell cycle,[193] while overexpression produced a greater transition into the S, G2, and M phases.[194] Indeed, c-Jun is crucial in the regulation of the G1/S phase transition.[195] c-Jun also plays an important role in both inducing and inhibiting apoptosis.[196] Endogenously, c-Jun inhibits the expression of apoptosis-inducing genes and maintains cell survival, particularly p53 [196,197]. However, JNK signaling can cause c-Jun to induce apoptosis via survival factor removal.[198] JNK phosphorylates the Ser63 and Ser73 residues of the c-Jun activation domain, causing AP-1 transcriptional activity to increase.[199] This can cause the induction of apoptosis, although AP-1 is also involved in the inhibition of apoptosis as well depending on the tissue and developmental stage.[196] As one might assume, c-Jun, along with JNK has been implicated in many cancers.[185,197]

CapZ-interacting protein (CapZIP)

CapZIP is a protein detected in muscle extracts that interacts with the F-actin capping protein CapZ. Capping proteins normally inhibit actin polymerization, preserving the actin monomer pool.[200] In humans, CapZIP is phosphorylated at Ser-179 and Ser-244 by mitogen-activated protein kinase-activated protein kinase 2 (MAPKAP-K2).[45] CapZIP serves as a substrate for stress-activated protein kinases (SAPKs), which can also phosphorylate it at several sites (Ser-68, Ser-83, Ser-108, and Ser-216).[45] Cell exposure to various stressful events causes the activation of several MAPKs, including SAPKs, JNK1, and JNK2. When under stress, SAPK3 and SAPK4 will phosphorylate CapZIP, triggering the dissociation of CapZIP from CapZ and enabling CapZ to modify actin filaments.[45] JNK1 can also phosphorylate CapZIP, although slower and less extensively.[45] Northern blot analysis reveals that CapZIP is expressed highly in skeletal muscle but to a lesser extent in cardiac muscle. In other organs such as the brain, lung, and liver, it is hardly detectable at all.[45] Multiple-tissue expression arrays revealed that CapZIP is expressed in immune organs like the thymus, spleen, lymph nodes, and bone marrow.[45] CapZIP is also expressed in immune cells and has been isolated from B cells, as well as leukemia and lymphoma cell lines.[45] CapZIP also regulates ciliogenesis via its upstream regulator Dvl. Dvl binds to ERK7 and CapZIP, functioning as a scaffold for a MAPK family member, ERK7 (also called MAPK15), to phosphorylate CapZIP, enabling ciliogenesis.[201]

Myosin II regulatory light chain (MRLC)

MRLC is a regulatory component of myosin II. Myosin light chain kinase (MLCK) phosphorylates the Ser19 residue of MRLC, and sometimes can even phosphorylate the Thr18 residue.[202] This enhances the activity of the actin-activated Mg^{2+} ATPase in myosin II, increasing the assembly and stability of myosin II filaments. The dephosphorylation of MRLC increases myosin II activity and stability more so than monophosphorylation.[203,204] However, both forms of MRLC are required for organizing stress fibers during interphase and forming the contractile ring in cell division.[202] Dephosphorylation is also important for the disassembly of

previous myosin filaments to form new ones.[205] ROCK1 can phosphorylate MRLC to facilitate the generation of traction for cell motility.[206] ROCK proteins also dephosphorylate MRLC,[205] enabling the colocalization of MRLC with actin filaments in mitotic and interphase cells.[202] Diphosphorylated MRLC also induces the formation of thick actin bundles containing myosin II, while unphosphorylated MRLC inhibits actin bundle formation.[202] These findings emphasize the importance of MRLC in actin organization.

Diaphanous 1 (DIA1)

DIA1 (also called mDia in mammals) is a member of the diaphanous-related formins (DRFs) that are Rho-GTPase binding proteins.[46] DIA1 contains a novel formin homology (FH) 2 domain that protects the barbed end of the actin filament from capping proteins and enables rapid assembly of actin subunits.[207] DIA1 also has an FH1 domain that binds profilin-bound G-actin to bring it closer to the barbed end for elongation of the actin filament.[207,208] DIA1 contains a Rho-GTPase binding domain in the N-terminal region and a Diaphanous-autoregulatory domain (DAD) in the C-terminal region. The N-terminal regulatory region is usually bound to DAD, causing autoinhibition. However, the binding of RhoA relieves this inhibition.[209,210] DIA1 is involved in a variety of processes, such as mechanotransduction, cell polarity, migration, and even exocrine vesicle secretion.[47] DIA1 mutations have been associated with deafness, cancer, and mental retardation.[47] Mutations in formins like DIA1 have also been implicated in cancer metastasis due to the loosening of cellular adhesion. Expression levels of DIA1 have correlated with the stage and metastasis of cancer cells.[210]

Intracellular mediators of the Wnt/Ca^{2+} pathway

Phospholipase C (PLC)

PLC is activated by G protein due to Wnt ligand binding in the Wnt/Ca^{2+} pathway.[52,53] The PLC family contains 13 different members with various functions and different structures. Although these members usually have little amino acid sequence homology, they have conserved EF-hand domains, PH domains, and C2 domains.[211] They also all have catalytic X and Y domains.[211] The PH domain is located towards the N-terminus and mediates the recruitment of PLC to the plasma membrane via binding to PIP2.[212] The EF-hand motifs are part of the catalytic core of PLC along with X, Y, and C2 [213]. The EF-hand motif undergoes a conformational change upon binding of Ca^{2+} to PLC, revealing binding sites for other ligands.[214] The X and Y domains form a triosephosphate isomerase (TIM) barrel-like structure composed of alternating α- and β-pleated sheets.[213] The X domain contains all the catalytic residues, while the Y domain is important in modulating the preference of PLC for PIP2, as well as two other ligands PIP and PI.[215] The C2 domains are formed from an eight-stranded β-pleated sandwich.[213] Upon Ca^{2+} binding, C2 domains can mediate PLC binding to phospholipids to mediate signal transduction and membrane trafficking.[216] Hokin et al used P^{32} to detect how phospholipid levels changed during enzyme secretion in pancreas slices with the addition of acetylcholine or carbamylcholine. They discovered that phospholipid activity increased five-to nine-fold.[217]

Unbeknownst to them at the time, the enzyme responsible for the increased phospholipid level was PLC.[214] PLC cleaves PIP2 into IP3, which can then induce the intracellular release of Ca^{2+}.[218] PLC can be activated by a wide variety of receptors, such as B-cell receptors, T-cell receptors, Fc receptors, tyrosine kinase receptors, and G-protein coupled receptors. Due to the variety of receptors, this means that PLC can be activated by a wide variety of ligands, such as neurotransmitters, hormones, and histamine.[214] Aside from IP3, the other product of PIP2 cleavage is DAG, which serves as a secondary messenger to activate Ca^{2+}-dependent protein kinase C (PKC) to phosphorylate numerous downstream effectors and activate a wide array of cellular functions, such as cell polarization, proliferation, as well as learning and memory.[219,220] Through Ca^{2+} release, PLC can regulate cell proliferation, differentiation, motility, gene expression, and other functions.[214]

Protein kinase C (PKC)

PKC is a family of protein kinases involved in a wide variety of diseases, such as diabetes, cancer, and heart disease.[221] There have been 518 protein kinase genes identified.[222] PKC, in particular, phosphorylates Ser and Thr residues.[221] PKC is activated by DAG, but can also be activated by phorbol esters, which are tumor promoters that mimic the action of DAG.[221,223] Specifically, DAG and phorbol esters bind to the C1 domain on PKC, which contains a cysteine-rich sequence that resembles a DNA-binding zinc finger domain.[224] PKC contains a regulatory region in the N-terminal half and a catalytic region in the C-terminal half. The C1 and C2 domains are located in the regulatory region and bind to the catalytic region, inhibiting its activity.[221] The catalytic kinase region contains C3 and C4 domains.[221] An important aspect of PKC is that, upon activation, it translocates to various cellular locations, joining with specific anchoring proteins at each site of action.[225] With phorbol esters that irreversibly activate PKC isoenzymes in a nonselective manner, PKC was discovered to regulate many cellular functions.[221] Some of these include cell proliferation, cell death, regulation of ion channels and receptors, cell-to-cell contact, and increasing gene transcription.[221] However, this early work using phorbol esters does not reflect the effects of DAG due to the irreversibility of phorbol ester binding. The lack of selectivity of phorbol esters also means that they cannot identify the function of each PKC isoenzyme.[221] Isoenzyme-specific inhibitors are still under development and undergoing trials, but this endeavor has proven to be difficult.[221,223]

Cdc42 protein

Cdc42 is a member of the Rho family of small GTPases with roles in actin cytoskeleton regulation, cell motility, cell polarity, and cell cycle progression among other functions.[226] Cdc42 was characterized in *Saccharomyces cerevisiae*, a species of yeast, with G25K being its mammalian and human homolog.[227,228] Cdc42 has a P-loop, two switch regions (switch I and switch II), a polybasic region at the C-terminus, and a CAAX box for posttranslational geranylgeranylation.[226] Cdc42 has been found in distinct pools in the Golgi apparatus, ER, and plasma membrane.[229,230] The Golgi pool functions in three ways; it serves as a reservoir of Cdc42, functions independently from the

plasma membrane pool to control protein transport from the Golgi apparatus, and coordinates with the pool to dictate cell polarity.[231] Cdc42 modulates the Golgi-to-ER transport via actin regulation.[232] The plasma membrane pool serves to regulate cell polarity via actin cytoskeletal rearrangement.[233] At the ER, Cdc42 is necessary for tubule fission during ER remodeling.[234] The activation of Cdc42 is regulated by GEFs, but unlike other Rho GTPases, Cdc42 is a hydrolase that can hydrolyze the GTP to GDP in the presence of GAP, even though GAP normally exchanges GDP for GTP.[235,236] GDI inhibits Cdc42 like other Rho GTPases, via preventing GDP/GTP exchange.[174] However, GDI also functions as a chaperone, delivering Cdc42 to its proper location and preventing its degradation.[230] Cdc42 is currently under investigation as a therapeutic target for the treatment of cancer, although there are few Cdc42 mutations and no driver mutations that have been linked to cancer.[226]

Ca^{2+}/calmodulin (CAM)-dependent kinase II (CAMKII)

CAMKII is a Ser/Thr protein kinase[237] that was first characterized as a Ca^{2+}-dependent regulator by Schulman and Greengard in 1978.[238] There are over 80 known CAMKs.[222] CAMKII is encoded by four different genes (α, β, γ, δ) in eukaryotes.[239] CAMKII monomers form 12 subunit holoenzymes, with a C-terminal association domain that brings the N-terminal catalytic kinase domains together to fold into two rings of six subunits each.[237,239] There is a regulatory segment that follows the kinase domain and is joined to a linker region that then connects to the association domain.[237,240] The regulatory domain forms an α-helix to block the catalytic domain of each subunit.[237] Additionally, the T286 phosphorylation site is sequestered in a hydrophobic groove, preventing autophosphorylation that would up-regulate CAMKII activity. Upon Ca^{2+}/CAM binding to the regulatory domain, the regulatory segment is removed, enabling progressive autophosphorylation of the T286 site and increasing CAMKII activity.[237] Normally, in the autoinhibited (compact) state, CAM cannot bind[237,240,241]; it is only when the compact state is in equilibrium with the non-autoinhibited (extended) state that CAM can bind the regulatory domain.[237]

Between the four CAMKII genes found in humans, the enzymes produced share 95% of their amino acid sequences in the kinase domains and 80% homology in the hub domains, with the linker region being the primary variable component.[240] When two adjacent subunits are activated by Ca^{2+}/CAM, they can phosphorylate one another on Thr286, causing increased Ca^{2+}-independent activity.[240]

CAMKII has a role in adaptive contractive response during aerobic exercise.[242] It is also involved in glucose production, cell cycle progression, and vascular smooth muscle function.[243] Continuous activation of CAMKII can cause cardiac myocyte apoptosis, heart failure, and cardiac arrhythmia.[244] CAMKII has also been identified as being able to spread the inflammatory response caused by damage to heart muscle.[243] The progression of cardiomyopathy and even Chagas disease caused by *Trypanosoma cruzi* is mediated by CAMKII signaling.[243,245]

TGF-β activated kinase 1 (TAK1)

TAK1 is a member of the MAPK kinase kinase (MAPKKK) family. It is a Ser/Thr kinase that was originally discovered to be a mediator of BMP and TGF-β signaling.[246,247] TAK1 can be activated by many cytokines, such as TNF-α, TGF-β, TLRs, and IL-1 [248,249]. TAK1 activation causes the phosphorylation of TAK1, which leads to the activation of NF-kB, JNK, ERK, p38, and MAPKs.[248,249] TAK1 is also involved in T- and B-cell signaling,[248] as well as angiogenesis during embryonic development.[249] Due to its role in immune and inflammatory processes, TAK1 has been implicated in multiple cancers, such as lymphoma and neuroblastoma, as well as colon, ovarian, and pancreatic cancers.[250] In addition, a blockade of TAK1 leads to p53 up-regulation, indicating the importance of TAK1 in controlling cellular stresses.[251] Activation of TAK1 requires three proteins, TAK1-binding protein 1 (TAB1), TAB2, and TAB3. TAB1 serves as an adaptor protein located on the N-terminal kinase domain of TAK1, while TAB2 and TAB3 will only bind the C-terminal TAK-binding domain after stimulation.[249] TAB1 overproduction leads to increased TAK1 activity, but TAB1 deficiency has minor downstream effects.[249] TAB2 and TAB3 are not required early on in TAK1 activation but are required for sustained TAK1 activation.[249] In mice, TAK1 has been demonstrated to be a regulator of TNF signaling in the skin and modulates skin inflammation. It was found that a lack of TAK1 caused keratinocyte death due to absent NF-kB and JNK-mediated cell survival signaling.[252] Recently, a selective TAK1 inhibitor called Takinib has been developed; it is activated by ATP and competitively inhibits TAK1 by binding to its ATP-binding pocket.[253] Another TAK1 inhibitor called piperidylmethyloxychalcone (PMOC) also functions in the same manner.[254]

Nemo-like kinase (NLK)

NLK is a conserved Ser/Thr MAPK.[255] It is the mammalian homolog of the *Drosophila* nemo gene, which was identified in *Drosophila* as important for the rotation of photoreceptor clusters in eye morphogenesis.[256] NLK has a longer N-terminal region that is rich in histidine, proline, alanine, and glutamine.[257] NLK can phosphorylate TCF4 to prevent the β-catenin/TCF complex from binding to DNA and initiating gene transcription. TAK1 can also stimulate NLK to phosphorylate TCF4, so NLK functions as a downstream effector of TAK1 in the repression of Wnt/β-catenin signaling.[58] Although TAK1 and NLK can repress canonical Wnt signaling, CAMKII from the noncanonical Wnt/Ca^{2+} pathway activates TAK1 in order to do so, indicating crosstalk between the two pathways.[57] Furthermore, TAB2 can serve as a scaffold for TAK1 and NLK, enabling cooperative interaction for the inhibition of canonical Wnt signaling.[258] NLK can also associate with NLK-associated RING finger protein (NARF), which is an E3 ubiquitin ligase whose action is up-regulated by NLK kinase activity.[259] NARF can ubiquitinate TCF/LEF for degradation by the proteasome.[259] NLK is involved in other pathways as well, such as Notch signaling and STAT protein signaling.[259] Due to the function of NLK in Wnt signaling and other pathways, it is crucial in regulating cell proliferation, apoptosis, migration, and other functions.[259] For instance, NLK was found to inhibit the growth and migration of non-small cell lung cancer by restoring the expression of E-cadherin, which normally suppresses migration and invasion.[260] However, analysis of colorectal cancer (CRC) cells found that NLK activity was increased, and only half of the CRC

cells were apoptotic compared to non-CRC cells, indicating that NLK may have an anti-apoptotic function.[261] NLK has also been identified as a pathological effector in mouse hearts, leading to progression toward heart failure and other cardiac conditions.[262] This makes NLK a potential therapeutic target for various diseases.

Calcineurin (CaN)

CaN is a conserved Ser/Thr phosphatase that is activated by increased intracellular Ca^{2+} [263]. It was first discovered by Wang and Desai in 1976 as a protein that counteracted the activation of bovine brain cyclic nucleotide phosphodiesterase.[264] CaN is a heterodimer consisting of calcineurin A (the catalytic subunit) and calcineurin B (the regulatory subunit).[265] The catalytic domain of calcineurin A is towards the N-terminal end, while the C-terminus contains three regulatory domains, the calcineurin B binding domain, the calmodulin-binding domain, and the autoinhibitory domain that binds to the active site when Ca^{2+}/calmodulin is absent.[265] Calcineurin B contains four Ca^{2+} binding EF-hand motifs.[265] The mechanism of calcineurin B activation is still unknown.[266] It is also structurally homologous to CAM.[263] CaN along with CAM binds to Ca^{2+}, then CAM binds CaN. This complex is what forms the active phosphatase.[263] Although there are multiple CAM-modulated kinases, CaN is the only phosphatase that is directly activated by Ca^{2+} [263]. CaN is best known for regulating the transcription of IL-2, dephosphorylating the transcription factor NF-ATp in response to an increase in intracellular Ca^{2+} via T cell receptor activation, and stimulating NF-ATp. CaN also controls cellular Ca^{2+} sequestration and cytokinesis.[266] The immunosuppressive drugs FK506 and cyclosporin A can both target CaN and were key to identifying its functions.[266] CaN is also important in the regulation of neurotransmitter release in neuromuscular junctions (NMJs).[267] Hypertrophic and dilated cardiomyopathy have both been linked to CaN overactivation.[268]

Antagonists and inhibitory regulators of Wnt signaling

Extracellular antagonists of Wnt signaling

Secreted frizzled-related proteins (sFRPs)

The largest family of secreted Wnt inhibitors are the sFRPs, which are structurally like the CRD ligand-binding domain of the Fz receptors.[269] The first sFRP discovered was the Frizzled motif associated with bone development (Frzb).[270] The sFRPs are about 295–346 amino acids long, with a CRD at the N-terminus.[269] These CRDs share 30%–50% sequence similarity with the CRDs of Fz receptors, with 10 cysteine residues linked by disulfide bridges. The C-terminus has a netrin-related motif (NTR) that functions as a heparin-binding domain.[271] Studies of Xenopus embryos revealed that Frzb binds to Wnt1 and Wnt8, sequestering them away from the Fz and LRP5/6 receptor complex.[272–274] sFRPs can also form a nonfunctional complex with Fz receptors to prevent Wnt binding and signaling.[275,276] Both the CRD and NTR domains are important in Wnt inhibition.[277] The CRD domain binds to Wnt,[273] while the NTR domain mimics the function of the entire sFRP1 to bind to Wnt ligands and inhibit Wnt signaling.[278]

Wnt-inhibitory factor 1 (WIF-1)

WIF-1 was first identified as an expressed sequence tag in the human retina[279] found in fish, amphibians, and mammals.[279] It contains a 150 amino acid long N-terminal signal sequence called the WIF domain, five epidermal growth factor (EGF)-like repeats, and a 45 amino acid hydrophilic domain at the carboxy-terminus.[279] The complete WIF-1 protein is 379 amino acids long.[279] Analysis of Xenopus embryo assays found that WIF-1 binds Wnt8, while in *Drosophila* clone-8 cells, WIF-1 binds Wg, although not as strongly as with Wnt8.[279] WIF-1 inhibits the binding of Wnt8 to Dfz2, blocking Wnt signaling.[279]

Dickkopf (Dkk) proteins

The Dickkopf (Dkk) proteins contain four members in vertebrates (Dkk1-4). Dkk1 was the first protein of its family to be discovered through experiments determining its importance in embryonic head formation and as a Wnt antagonist.[280] The Dkk proteins are glycoproteins composed of 255–350 amino acids.[281] Interestingly, although the Dkks all have a signal sequence and two conserved CRDs, there is little sequence similarity between them otherwise.[280] Dkks primarily modulate the canonical Wnt signaling pathway via binding to the LRP5/6 co-receptor. Dkk1 associates with one of two single-pass transmembrane proteins called Kremen 1 or 2 (Krm1 or Krm2) while binding to LRP5/6. This interaction allows the endocytosis of the LRP5/6 receptor.[19,281] Analysis of Dkk1 in cardiogenesis suggests that Dkk1 can activate JNK, indicating that Dkk1 may be involved in the noncanonical Wnt/PCP pathway as well.[282]

Wise and sclerostin (SOST) proteins

Wise and SOST are both members of a subfamily of cysteine knot proteins as they both contain cysteine knot motifs.[283] Both Wise and SOST are also BMP antagonists.[283,284] Wise protein is composed of 206 amino acids with 38% sequence homology with sclerostin.[284] Wise is expressed in a wide variety of tissues, including branchial arches, rat endometrium, developing testes, and more.[285] The sclerostin polypeptide is about 190 amino acids long with a cysteine knot formed from its flexible N- and C-terminal regions.[286] Sclerostin is expressed in osteocytes to regulate bone formation. Deletions in the SOST gene that codes for sclerostin can result in van Buchem disease, which is characterized by a high bone mass due to loss of inhibition of bone formation.[286] Reporter assays demonstrate that Wise can block Wnt1, Wnt3a, and Wnt10b.[285] Wise also binds to the LRP6 coreceptor, preventing the binding of Wnt8.[284] Additionally, Wise can function intracellularly to prevent LRP6 trafficking to the cell surface.[287] Wise can also bind LRP4 to inhibit Wnt signaling.[288] Sclerostin binds LRP5/6, specifically binding to LRP5 at the YWTD-EGF repeat domains, inhibiting Wnt signaling.[289,290]

Cerberus

Cerberus is an abundant organizer-specific gene that was isolated from Xenopus and can induce ectopic head formation.[291] It also plays an important role in cardiogenesis during vertebrate embryonic development.[292] Cerberus,

like Wise and SOST, is in the cysteine knot superfamily.[293,294] Long-form Cerberus (xCer-L) binds to Wnt8 and could inhibit Wnt signaling, but short-form Cerberus (xCer-S) cannot do the same.[294] Further study is required to better understand the effects of Cerberus on the Wnt pathway.

IGFBP-4

IGFBP-4 is a member of the insulin-like growth-factor-binding proteins (IGFBPs) that bind and modulate insulin-like growth factors (IGFs). IGFBP-4 is important in cardiomyogenesis *in vitro*.[295] IGFBP-4 interacts with LRP6 and Fz8, functioning as a competitive inhibitor of Wnt3a binding.[295] In this manner, IGFBP-4 inhibits the canonical Wnt pathway.[295,296] Furthermore, there are six IGFBP members. Although IGFBP-4 is the most powerful Wnt inhibitor, IGFBP-1, -2, and -6 also have Wnt inhibitor activity, albeit modestly. On the other hand, IGFBP-3 and -5 have no Wnt inhibition activity.[295] Although some cancer cell lines *in vitro* saw a decrease in cell proliferation when treated with IGFBP-4, decreased levels of the protein increased the risk of breast cancer, and overexpression of IGFBP-4 caused prostate cancer growth *in vivo*.[295]

Intracellular negative regulators of Wnt signaling

Adenomatous polyposis coli (APC)

APC is a tumor suppressor gene that is heavily associated with colorectal cancer (CRC).[297] The APC protein is 2843 amino acids long,[297] with a central region spanning about 1000 amino acids containing motifs that bind β-catenin or Axin.[22] In humans, APC contains four 15-mers and seven 20-mers. The 20-mer repeats are phosphorylated by GSK-3β and CK1, enhancing the affinity of APC for β-catenin.[22] Interspersed with the 20-mer repeats are three Ser-Ala-Met-Pro (SAMP) repeats that bind to Axin.[298,299] Full-length APC or APC with SAMP repeats was found to protect β-catenin from dephosphorylation by PP2A, ensuring the destruction of β-catenin.[299] The N-terminus of APC has a dimeric coiled-coil domain, with a heptad-repeat region that is crucial for the dimerization of APC.[300] APC also contains an armadillo repeat domain (APC-arm) that enables APC to regulate the actin cytoskeleton and microtubules during cell polarization and migration.[301,302] The C-terminal region of APC binds several proteins, such as microtubulin, indicating its role in microtubule assembly.[303] APC mutation is best known for causing familial adenomatous polyposis (FAP), a disease characterized by a family history of colorectal polyps and cancer. FAP is inherited in an autosomal dominant manner via a germline mutation.[304]

Axin proteins

Axin is a scaffold protein that was initially discovered as a protein product of the mouse Fused (Fu) gene, inhibiting Wnt signaling and regulating embryonic axis formation.[305] There are two Axin homologs in eukaryotic organisms, Axin1 and Axin2, which are often collectively referred to as Axin. Axin1 is vital for embryonic viability and is widely expressed, while Axin2 is limited in its distribution to certain tissues.[306,307] Axin1 was identified in mice as a locust causing kinky tail phenotypes, while Axin2 was identified due to its interactions with GSK-3β and β-catenin, as well as its homology to Axin1. The N-terminus contains the RGS (regulation of G-protein signaling) domain, which binds to APC.[298] It is important to note that this region is homologous to regulators of G-protein signaling, but does not actually regulate any known G-protein.[298] The RGS domain binds the third SAMP repeat and is highly conserved among Axins but is not conserved in other RGS proteins.[298] Axin contains a C-terminal DIX domain that enables it to form homodimers with other Axin proteins, as well as heterodimers with Dvl.[308,309] This interaction via the DIX domains is how Dvl can inhibit Axin and enable transduction of Wnt/β-catenin signaling.[144] In between the RGS and DIX domains are regions that allow Axin to bind to β-catenin, GSK-3β, and CK1, forming a complex that will target β-catenin for degradation.[307] Axin is important in many developmental processes, including anterior—posterior axis formation, organogenesis, neuronal proliferation and differentiation, synapse formation, and several others.[307] Both Axin1 and Axin2 can function as the scaffold of the β-catenin destruction complex. They share the RGS and DIX domains, as well as the Tankyrase binding domain that maintains the Axin protein's stability.[306] Interestingly, Axin2 is a target of β-catenin mediated gene transcription, but not Axin1. Due to this fact, Axin2, in particular, has been of interest in cancers caused by aberrant Wnt signaling activation, which leads to high levels of Axin2 expression.[306] Axins also interact with many other pathways. Its interactions with p53 are important in stimulating transcription in p53-dependent target genes, shedding light on the importance of Axin as a tumor suppressor.[310] Axin1 and Axin2 both inhibit Wnt signaling and stimulate TGF-β signaling. However, TGF-β signaling can inhibit Axin1 and Axin2 expression, leading to enhanced Wnt signaling that increases chondrocyte maturation.[311]

Glycogen synthase kinase-3 (GSK-3) proteins

GSK-3 is a conserved Ser/Thr kinase that was first identified in rabbit skeletal muscle.[312,313] There are two forms of GSK-3 in mammals, GSK-3α and GSK-3β, which are 98% homologous in the internal kinase domain, albeit with different N-terminal regions[314] and C-terminal regions.[315] Defects in GSK-3α in mice caused impaired locomotion and coordination, as well as psychiatric disorders.[316] GSK-3β defects, on the other hand, are embryonically lethal,[317,318] indicating that GSK-3β may be more important than GSK-3α. GSK-3 inhibits glycogen synthase via phosphorylation, thereby inhibiting glycogen synthesis.[319] GSK-3 is inactivated by insulin, so GSK-3 dysregulation has been implicated in type II diabetes.[318] Since both Wnt and insulin signaling pathways involve GSK-3β, this suggests that there is potential crosstalk between them. AKT phosphorylates and inhibits GSK-3 [320] and Wnt causes GSK-3 dislocation from the destruction complex.[321] GSK-3β is rich in the brain and is involved in neurogenesis, neurotransmission, and regulation of synaptic plasticity.[322] However, it has also been implicated in the formation of neurofibrillary tangles in Alzheimer's disease.[322] GSK-3β can be inhibited by lithium, which is used as a mood stabilizer for treating bipolar disorder.[323] GSK-3β also enhances apoptosis via up-regulation of p53.[324] GSK-3 can also modulate inflammation downstream of TLR signaling; activation of GSK-3 leads to the

production of the pro-inflammatory cytokines IL-6, IL-1B, and IFNy, while inhibition of GSK-3 leads to the production of anti-inflammatory IL-10.[325]

Casein kinase 1 (CK1)

Casein kinase 1 (CK1) is a large family of conserved Ser/Thr kinase found in eukaryotes, with seven isoforms in humans (α, γ1, γ2, γ3, δ, ϵ, and α-like).[326] CK1 is involved in a diverse range of cellular processes, such as vesicular trafficking, DNA repair, cell proliferation, and apoptosis.[327] The kinase domain is conserved, but the N- and C-terminal domains vary in length among the CK1 family members.[326] The N-terminal domain of CK1 is its catalytic domain, while the C-terminal domain has been associated with an inhibitory function, as CK1 with a truncated C-terminus led to an increase in catalytic domain activity.[328,329]

CK1 is generally considered constitutively active.[330] The reason is unknown, but phosphatases have been inferred to be involved in this activity.[329,330] Phosphorylation of CK1 either by itself or by other kinases inhibits its catalytic activity.[330] Autophosphorylation on the C-terminus results in a phosphopeptide that binds to the catalytic domain.[330] CK1 isoforms are regulated by scaffold proteins, which can sequester CK1 at several subcellular locations or bind CK1 allosterically to promote or inhibit CK1 activation.[330] Although CK1 is also involved in the Hedgehog, NF-kB, and Hippo signaling pathways,[326,327,330] Wnt signaling is perhaps its best-characterized process.[330] CK1 phosphorylates β-catenin on Ser45, priming it for subsequent phosphorylation on Thr41, Ser37, and Ser33 by GSK-3.[331] β-TrCP can then ubiquitinate β-catenin for proteasomal destruction.[22,24]

CK1 also has a role in modulating p53 due to its inhibitory interacting proteins, mouse double minute homologue 2 and 4 (MDM2 and MDM4), being substrates of CK1. Phosphorylation of these substrates by CK1-δ and CK1-ϵ leads to p53 activation.[332] However, other studies have demonstrated that the knockdown of CK1-α can also activate p53.[333] This suggests that different isoforms could have opposite effects on p53 signaling. CK1 was also the first kinase discovered to regulate the circadian rhythm, where the positive regulatory complex CLOCK-BMAL activates the transcription of PER orthologs PER1-3, and cryptochrome proteins CRY1-2. The PER and CRY proteins then inhibit the CLOCK-BMAL complex.[330] This completes the circadian cycle, but then CK1 can phosphorylate PER proteins for degradation, starting the cycle again.[330] Additionally, CK1 is involved in mitosis, regulating spindle positioning upon being delivered to spindles by the CK1-specific binding protein FAM83D.[334] CK1 has been prominently linked to neurodegeneration and cancer.[330]

β-Transducin repeat-containing proteins (β-TrCP)

β-TrCP is the substrate recognition subunit for the Skp1/Cullin1/F-box protein (SCF) E3 ubiquitin ligases.[335] β-TrCP (also called FWD1 in mammals) is part of the Fbw (F-box/WD40 repeat containing) protein family, whose members share an N-terminal F-box motif and seven C-terminal WD40 repeats. Other F-box proteins include Fbl (F-box/leucine-rich repeats) and Fbx (F-box/unknown motifs).[335] These motifs are highly conserved, and humans have two isoforms, β-TrCP1 and β-TrCP2.[335] β-TrCP functions as an intracellular receptor to bind phosphorylated β-catenin at the phosphorylated sites, but this interaction is more efficient in the presence of Axin.[336] In addition, Axin does not bind well with β-catenin without β-TrCP, suggesting that the three form a ternary complex that requires all three members for optimal stability.[336] The F-box motif is used to bind β-TrCP to Skp1, which can then ubiquitinate β-catenin for degradation.[337] However, the F-box domain is not required to bind β-catenin, as the binding to β-catenin and Axin is mediated by the WD40 repeat domains.[336]

SCF uses β-TrCP to determine substrate specificity.[336] SCF ubiquitinates substrates like β-catenin at DSGXXS destruction motifs, with both Ser residues needing to be phosphorylated for SCF-mediated ubiquitination.[335] β-TrCP/SCF is also involved in the NF-kB signaling pathway. NF-kB is usually retained in the cytoplasm by a family of inhibitory molecules called IkBs. These can then be phosphorylated by IkB kinase (IKK) and subsequently ubiquitinated by SCF for degradation, releasing NF-kB to translocate to the nucleus.[338] β-TrCP has been implicated in cell division due to β-TrCP knockout mice having impaired progression through mitosis, with spermatocytes accumulating at metaphase I.[339] This effect is attributed to the buildup of early mitotic inhibitor 1 (Emi1), which inhibits the activity of the anaphase-promoting complex/cyclosome (APC/C).

Normally β-TrCP/SCF would degrade Emi1 to allow progression through mitosis, but this does not happen without β-TrCP recognizing the DSGXXS destruction motif.[340] Due to the oncogenic risks of NF-kB in inducing gene products controlling signaling proliferation and suppressing apoptosis,[341] β-TrCP/SCF is linked to oncogenesis and anticancer therapy resistance.[335] β-TrCP/SCF is also under investigation for targeting disease-causing proteins for degradation.[335]

Protein phosphatase 2A (PP2A)

PP2A is a member of the phosphoprotein phosphatase (PPP) family and is involved in cell cycle regulation.[342] It is a Ser/Thr phosphatase.[343] PP2A is a heterotrimeric enzyme consisting of A, B, and C subunits. A is the scaffolding subunit, B is the regulatory subunit, and C is the catalytic subunit.[343] The core of PP2A is composed of A and C subunits, which both have α and β isoforms that are homologous to each other, although the α isoform is the more commonly expressed variant.[343] The A subunit contains 15 huntingtin-elongation-A subunit-TOR (HEAT) tandem repeats, with each HEAT repeat consisting of a pair of antiparallel helices, which assemble to form a curved structure.[344] These helices form a hydrophobic inner ridge that facilitates B and C binding. They also stack with a hinge region between HEATs 12 and 13 to give the A subunit flexibility.[343] The catalytic activity of the C subunit is thought to be mediated by two Mn^{2+} ions,[343] coordinated by six conserved residues (two aspartate, one asparagine, and three histidine residues) along with a catalytic water molecule.[342] Modifications of the C-terminal tail of the C subunit are needed for B subunit binding, although the B subunit has different classes and each requires its own unique modification.[345]

There are four classes of B subunits, namely, the B55 family (B), the PR72 family (B′), the B56 family (B″), and the

Striatin family (B‴). They all vary widely in structure, allowing for diverse substrate specificity for a wide range of cellular functions.[343] Furthermore, although A and C subunits are expressed ubiquitously, B subunit expression levels vary significantly across different cells and tissues.[343] PP2A has dual roles in Wnt signaling. It can dephosphorylate β-catenin, APC, and Axin, enhancing Wnt signaling.[342] However, it also inhibits Wnt signaling by dephosphorylating the Ser9 residue on GSK-3β after being recruited by heat shock cognate 40 (HSC40), activating GSK-3β.[346]

PP2A can also activate GSK-3β indirectly by inhibiting protein kinase B (AKT), which initially phosphorylates GSK-3β to inhibit it.[320,346] Due to the different PP2A holoenzymes that exist, PP2A has a wide variety of functions. For instance, PP2A-B55 is crucial to the prevention of entry into mitosis and must be inactivated for cell division to begin.[342] However, PP2A holoenzymes are also involved in the disassembly and reassembly of the Golgi apparatus mediated by phosphorylation and dephosphorylation during mitosis. Specifically, dephosphorylation of the Golgi matrix protein GM130 by PP2A induces Golgi reassembly at the end of mitosis.[347] Mutations in PP2A have been associated with defects in brain development and are causally linked to intellectual disability and neurodevelopmental disorders.[348] Abnormalities in PP2A have also been linked to Alzheimer's disease.[349] It is also a factor in cancer onset, such as the progression of COPD in lung cancer when inactivated.[350]

Groucho

Groucho is a transcriptional corepressor that was first identified in 1968 by a mutation in *Drosophila* that caused clumps of extra bristles to form above the eyes. Transducin-like enhancer of split (TLE) protein is the human homolog.[351] TLE/Groucho is involved in a variety of processes, such as eye development, segmentation, and sex determination.[351] TLE/Groucho is characterized by a conserved glutamine-rich (Q) domain on the N-terminus, and a conserved WD-repeat domain on the C-terminus, with a less conserved central region dividing the two.[351] In the nucleus, TCF/LEF are bound to TLE/Groucho via the N-terminal glutamine-rich (Q) domain on TLE/Groucho.[352] The Q domain has an α-helical coiled-coil motif that enables TLE/Groucho to tetramerize, which is required for transcriptional repression.[352] The Q domain binds to the HMG domain of TCF/LEF.[352] The C-terminal WD-repeat domains function to interact with transcription factors and are also important in chromatin condensation.[353] TLE/Groucho also contains a central glycine/proline (GP)-rich domain that recruits histone deacetylases (HDACs) to repress transcription.[351,354]

TAK1 and NLK

As mentioned previously, from the noncanonical pathway, TAK1 and NLK can inhibit canonical Wnt signaling via TAK1 activation of NLK to phosphorylate TCF4.[58] NARF can also inhibit canonical Wnt signaling by ubiquitinating TCF/LEF for proteasomal degradation.[259] Additionally, the coexpression of the noncanonical Wnt ligand, Wnt5a, in hepatocellular carcinoma (HCC) cells, resulted in a three-fold decrease in TCF activity. However, the mechanism that caused this is unknown.[355]

Crosstalk between Wnt and major signaling pathways

Crosstalk with bone morphogenetic protein (BMP) signaling

BMPs are members of the transforming growth factor-beta (TGF-β) family of cytokines.[356] In TGF-β/BMP signaling, the binding of a ligand to type I and II receptors brings them together, where receptor II phosphorylates the receptor I kinase domain, propagating the signal via phosphorylation of the receptor-regulated Smad (R-Smad) signal transducing proteins.[357] Of the five R-smads (Smad1, -2, -3, -5, and -8), Smad1, Smad5, and Smad8 are activated by BMP signaling, while Smad4 is the co-mediator Smad (Co-Smad).[357] The R-Smads form complexes with Smad4 to translocate into the nucleus and regulate gene transcription.[357,358] BMPs are vital in embryogenesis and homeostasis and are known to induce bone formation.[11,356,359–363]

BMP receptors determine the strength of BMP signaling through the C-terminal phosphorylation on Smad1, which contains four GSK-3 phosphorylation sites. GSK-3 can phosphorylate Smad1, leading to its ubiquitination and destruction, which reduces the strength of BMP signaling. Wnt signaling can also inhibit GSK-3β, stabilizing Smad1 and enabling BMP signaling.[356] Smad7 is an inhibitory Smad, primarily down-regulating R-Smad activation via the recruitment of the E3 ubiquitin ligases: Smurf1 and Smurf2. Smurf1 and Smurf2 target Smads and the TGF-β receptors for degradation.[357] Smad7 can complex with β-catenin and Smurf2, which causes ubiquitination and subsequent degradation of β-catenin[364] (Fig. 5).

Additionally, Smad7 can interact with Axin, and this Smad7-Axin complex can cause GSK-3β and β-catenin to dissociate from Axin while also inhibiting the recruitment of Smurf2 to β-catenin. The overall effect is to stabilize β-catenin, which complexes with E-cadherin to strengthen cell-to-cell adhesion.[365] BMP receptor type 1a (BMPr1a) induces the expression of SOST to limit cancellous bone accrual by inhibiting Wnt signaling. However, BMPr1a-deficient osteocytes experience a decreased expression of SOST, indicating the role of BMPr1a in modulating bone growth through Wnt signaling.[358] Loss of BMPr1a in mouse rib bones also caused decreased levels of Dkk1, indicating Dkk1 serves as another downstream target of BMPr1a.[366] BMP9 induces the formation of ectopic bone via the recruitment of Runx2 and β-catenin to the osteocalcin promoter. Knockdown of β-catenin prevented osteogenic differentiation of MSCs via BMP9, establishing the importance of Wnt signaling in mediating BMP9 activity.[367]

Wnt3a and BMP9 both induce alkaline phosphatase (ALP) activity in MSCs while enhancing one another's ALP induction, suggesting that they act synergistically to induce bone formation.[367] Between the two, Wnt3a induces ALP activity to a stronger degree and earlier than BMP9, demonstrating the crosstalk between the two pathways in osteogenic differentiation.[367] Furthermore, Wnt3a and BMP9 both upregulate CTGF/CCN2, a gene involved in osteogenic differentiation.[363]

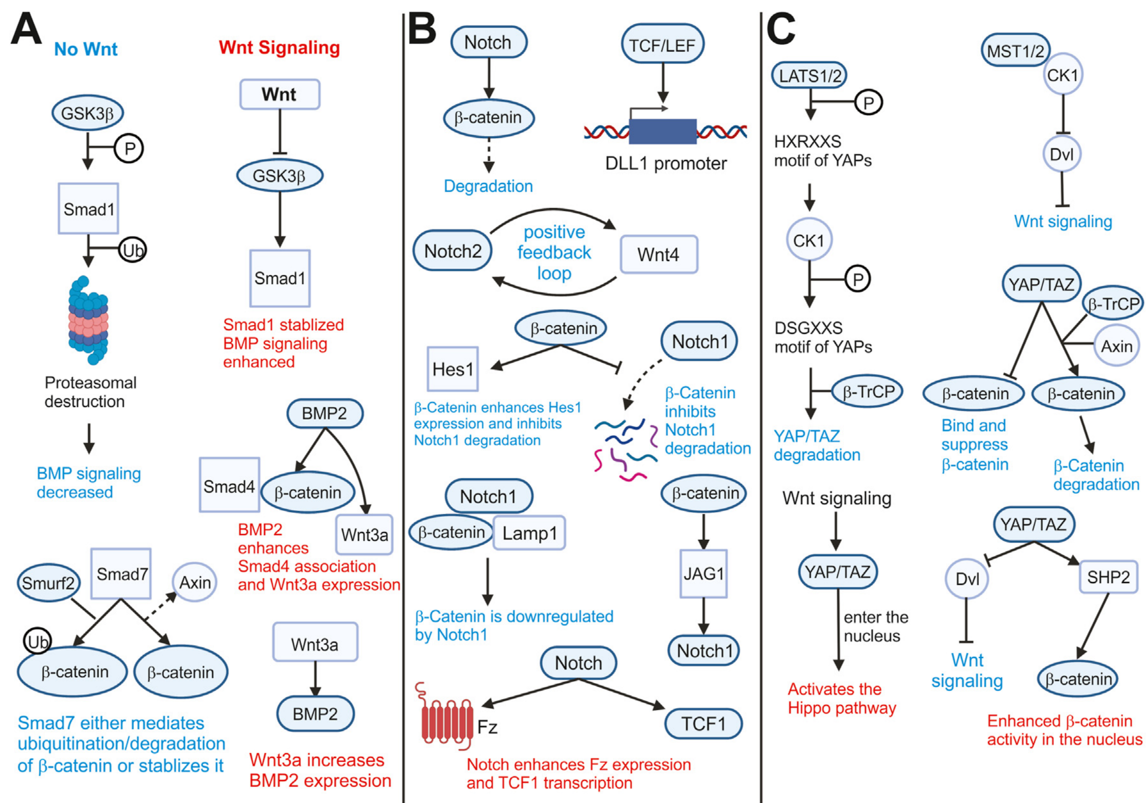

Figure 5 Crosstalk between Wnt signaling and other major signaling pathways. The **(A)** left, **(B)** middle, and **(C)** right panels show the Wnt/BMP crosstalk, Wnt/Notch crosstalk, and the Wnt/Hippo crosstalk, respectively. Note that not all crosstalk interactions between each of these pathways are represented in the image due to space constraints. In Wnt/BMP crosstalk, the absence of Wnt signaling allows GSK-3β to phosphorylate Smad1, which is ubiquitinated and degraded. The activation of Wnt signaling inhibits GSK-3β, stabilizing Smad1 and enhancing BMP signaling. BMP2 can up-regulate Wnt3a and enhance β-catenin binding with Smad4. Wnt3a can also up-regulate BMP2 expression. Smad7 can either recruit Smurf2 to ubiquitinate β-catenin for degradation or cause Axin to dissociate from β-catenin, stabilizing it. For Wnt/Notch crosstalk, Notch can complex with β-catenin and promote its degradation. TCF/LEF can regulate the expression of the DLL1 Notch receptor ligand. Notch2 and Wnt4 are involved in a positive feedback loop. β-Catenin can enhance Hes1 transcription and inhibit the degradation of Notch1. β-Catenin can also up-regulate transcription of the JAG1 Notch ligand. Notch1 can complex with β-catenin and Lamp1 to promote lysosomal degradation of β-catenin. Notch can increase both Fz receptor expression and transcription of TCF1. Finally, in Wnt/Hippo crosstalk, there are many interactions as well. LATS1/2 can phosphorylate the HXRXXS motifs of YAP to signal to CK1 to phosphorylate the DSGXXS destruction motifs, leading to the recruitment of β-TrCP-mediated degradation of YAP. MST1/2 can sequester CK1, preventing phosphorylation of Dvl and inhibiting Wnt signaling. YAP/TAZ can bind to and suppress β-catenin while preserving its stability, although other reports indicate that YAP/TAZ is associated with Axin as part of the destruction complex. YAP/TAZ can also inhibit Dvl, reducing Wnt signaling. However, YAP can also interact with SHP2 to enhance β-catenin activity in the nucleus. Wnt signaling can cause YAP/TAZ to transcribe Hippo pathway target genes. The illustration was inspired by and created in BioRender.

BMP2 stimulates LRP5 expression and inhibits β-TrCP expression. This leads to an increase in β-catenin levels in osteoblasts and promotes osteogenic differentiation.[368] BMP2 also increased the expression of the canonical Wnt ligands, Wnt1, Wnt3a, and Wnt4.[368] The BMP2-mediated up-regulation of Wnt3a and enhancement of the interaction of Smad4 with β-catenin reveal the importance of BMP2 in the modulation of Wnt signaling.[369] Interestingly, Wnt3a signaling or overexpression of β-catenin/TCF4 can increase transcription of BMP2, adding further complexity to the interplay of BMP and Wnt signaling.[370] BMP2 and Wnt3a exhibit a cooperative effect to increase the transcription of osteogenic genes (Id1, Dlx5, Msx2, Osx, and Runx2).[371]

BMP2 can also antagonize Wnt signaling by promoting Smad1 binding to Dvl1, preventing the activation of β-catenin.[372] BMP2, along with BMP4, can also induce Wnt8 expression in Xenopus mesoderm.[373] Perhaps unsurprisingly, mutations in BMP genes can lead to several bone disorders, such as skeletal developmental defects and inappropriate ossification.[374] BMP signaling has also been a therapeutic target in cardiovascular diseases such as atherosclerosis, pulmonary arterial hypertension, and anemia of chronic disease.[375]

Crosstalk with TGF-β signaling

TGF-β signaling coordinates with Wnt signaling in development and homeostasis. The presence of both signaling pathways is necessary for the initial inductive activity of

BMPs.[376] TGF-β and Wnt signaling regulate each other's ligand production, and the Smad/β-catenin/LEF complex regulates a group of shared target genes, further delineating the cooperativity of the two pathways.[356] Eger et al conducted a study of polarized mouse mammary cells that underwent epithelial—mesenchymal transition (EMT) induction by an inducible c-fos estrogen receptor (FosER) oncoprotein. The inhibition of both TGF-β and Wnt signaling caused a reversal of cells that underwent EMT back to their polarized forms, while inhibition of only one pathway led to a partial rescue of the epithelial features. This indicates the cooperativity of TGF-β and Wnt signaling in the EMT.[377] Furthermore, Nawshad et al demonstrated in palate medial-edge epithelial cells (MEE) that TGF-β3 signaling forms Smad2-P-Smad4-LEF1 transcription complexes that repress the E-cadherin gene during EMT.[378] The mediation of the EMT via β-catenin and TGF-β is dependent on cyclic AMP-responsive element-binding protein (CREB)-binding protein (CBP). One target gene in EMT is α-smooth muscle actin (α-SMA), which requires β-catenin for the induction of transcription via TGF-β signaling. CBP promotes the interaction between Smad3 and β-catenin after TGF-β1 activation, leading to the transcription of α-SMA. This signaling mechanism has been implicated in pulmonary fibrosis and is being explored as a therapeutic target.[379] TGF-β1 signaling also causes the translocation of β-catenin to the nucleus via Smad3 in MSCs. The stimulation of the TGF-β receptor causes phosphorylation of Smad3, disrupting its interactions with GSK-3β. Smad3 can then function as a chaperone to shuttle β-catenin into the nucleus.[380]

Crosstalk with Notch signaling

Notch signaling is a conserved cell—cell communication mechanism involved in determining cell fate and cell lineage.[381] In mammals, there are four Notch receptors (Notch1—4) that are homologous to the single Notch receptor in *Drosophila*, and five Delta/Serrate/Lag-2 (DSL) ligands (DLL1, DLL3, DLL4, JAG1, and JAG2).[382,383] Ligand binding leads to the opening of the negative regulatory domain (NRR). This enables the cleavage of the Notch intracellular domain (NICD) by the multiprotein γ-secretase complex. The NICD can then translocate to the nucleus and form a Notch transcriptional activation complex (NTC) with RBPJ and coactivators of the Mastermind-like (MAML) family. Without Notch, RBPJ interacts with transcriptional repressors, while the binding of Notch enables transcription.[382,383]

In *Drosophila*, Notch signaling promotes Wg expression at the wing margin, while Wg promotes Serrate and Delta expression in wing patterning. Notch has also been demonstrated to down-regulate Wnt signaling by complexing with β-catenin and promoting its degradation.[384] Notch2 is involved in a positive feedback loop with Wnt4, up-regulating Wnt4 expression to promote differentiation of progenitor cells in the nephrons of mice.[385] The Wnt pathway transcription factors LEF1/TCF regulate the expression of DLL1, establishing the importance of Wnt signaling in the regulation of Notch signaling.[386] Additionally, Wnt/β-catenin signaling also regulates the transcription of the Notch target gene Hes1, which encodes a basic helix-loop-helix transcriptional repressor.[387]

β-Catenin can bind to Notch1 and the NICD, which enhances the transcription of Hes1 while also preventing ubiquitin-dependent degradation of Notch1 and NICD.[388] On the other hand, Notch1 can down-regulate β-catenin by forming a complex with β-catenin and then colocalizing with the lysosomal protein Lamp1. Lysosomal activity can then decrease the levels of β-catenin.[389] GSK-3β phosphorylates NICD, stabilizing Notch and positively regulating its signaling activity.[390] CAMKII induced by Wnt5a signaling can phosphorylate the Notch signaling corepressor, silencing mediator or retinoic acid, and thyroid hormone receptor (SMRT). This causes SMRT to dissociate from RBPJ and get degraded by the proteasome. CAMKII also enhances binding between NICD and RBPJ. The overall effect of CAMKII is to increase Notch signaling.[391] Dvl down-regulates Notch signaling by inhibiting the CSL (RBPJ) transcription factors that mediate Notch signaling[392] (Fig. 5).

Notch-regulated ankyrin repeat protein (NRARP) is a negative regulator of Notch signaling but can also up-regulate Wnt signaling via stabilizing LEF1.[393] In both colorectal and renal cancers, Wnt/β-catenin signaling up-regulates transcription of the Notch ligand Jagged1 (JAG1), leading to Notch activation.[394,395] In hematopoietic progenitor cells (HPCs), Notch signaling up-regulates the expression of Fz receptors to enhance dendritic cell (DC) differentiation.[396] Notch signaling also increases transcription of TCF1, increasing mediation of Wnt signaling to stimulate T-cell specification.[397] Notch dysregulation can lead to kidney fibrosis and chronic kidney disease (CKD).[381] Defective Notch signaling can also cause congenital heart disease but has become a potential therapeutic target for heart regeneration.[398] Liver fibrosis, chronic liver disease, and osteosarcoma are also attributable to aberrant Notch signaling.[399,400]

Crosstalk with the Hippo/Yap pathway

The Hippo pathway plays an important role in regulating cell fate and tissue structure.[401] The Hippo pathway in *Drosophila* contains four core components, namely, the NDR family protein kinase Warts (Wts), the WW domain-containing protein Salvador (Sav), the Ste20-like protein kinase Hippo (Hpo), and the adaptor protein Mob as a tumor suppressor (Mats). The mammalian orthologs for each of these are LATS1/2 kinase (Wts), SAV1 (Sav), MST1/2 (Hpo), and MOB1 (Mats). MST1/2 forms heterodimers with SAV1 for the phosphorylation of SAV1, MOB1, and LATS1/2. This enables LATS1/2 to phosphorylate yes-associated protein (YAP) and WW domain-containing transcription regulator protein 1 (TAZ). YAP/TAZ are orthologs of the transcriptional coactivator called Yorkie (Yki), which normally stimulates transcription. The phosphorylation of YAP/TAZ by LATS1/2 and the Hippo kinase cascade inhibits transcription.[401] The Hippo pathway interacts with Wnt signaling in several ways.

Phosphorylation of the HXRXXS motif of Ser381 of YAP by LATS1/2 can provide a signal for CK1 to phosphorylate a phosphodegron, which is a DSGXXS destruction motif. This causes the recruitment of β-TrCP, which leads to YAP ubiquitination and eventual degradation, inhibiting YAP and preventing gene transcription.[402] TAZ is also degraded via

phosphorylation by CK1 in the phosphodegron and subsequent ubiquitination and degradation.[403] CK1 can also be bound by MST1/2, causing sequestration of CK1 and preventing it from phosphorylating Dvl, leading to inhibition of Wnt signaling.[404] An analysis of overexpression of YAP/TAZ in cells found that YAP/TAZ bound and suppressed β-catenin without affecting β-catenin levels, suggesting the preservation of β-catenin stability.[405] However, other reports indicate that when Wnt signaling is inactivated, YAP/TAZ normally binds β-catenin as part of the destruction complex. This association of YAP/TAZ to the destruction complex (via Axin, specifically) recruits β-TrCP, degrading β-catenin.[406] Further experimentation may be needed to determine whether YAP/TAZ affects the stability of β-catenin or not.

When Wnt signaling is activated, YAP/TAZ is released from this complex and moves to the nucleus to activate the Hippo pathway.[406,407] YAP/TAZ can also bind to Dvl, suppressing its phosphorylation and downstream effects on Wnt signaling.[405] YAP can also interact with Src homology 2 domain tyrosine phosphatase (SHP2), an amino acid phosphatase that enhances β-catenin activity in the nucleus. This enables YAP to dampen Wnt signaling strength.[407] These findings indicate that YAP/TAZ can serve as mediators or antagonists of Wnt signaling, where activation of Wnt signaling enables YAP/TAZ to transcribe Hippo pathway target genes, while inactivation leads to the antagonism of β-catenin.[405–407] The noncanonical Wnt5a/b and Wnt3a ligands can activate YAP/TAZ as mediators of noncanonical Wnt signaling.[408] Dysregulation of the Hippo pathway causes resistance to apoptosis and even chemotherapeutic treatments, leading to tumorigenesis, metastasis, and cancer relapse.[401] The inhibition of Wnt signaling by Hippo is also crucial in controlling heart size by limiting cardiomyocyte proliferation.[409]

Crosstalk with Hedgehog (Hh) signaling

The Hedgehog (Hh) pathway can be divided into canonical and noncanonical pathways. The canonical pathway is activated by the Shh ligand binding to the transmembrane protein Patched (Ptch1), which normally inhibits another transmembrane protein called Smo. The binding of Shh leads to Ptch1 degradation, causing Smo accumulation at the primary cilium (PC) which stimulates a signaling cascade. This leads to the Gli family of proteins translocating to the nucleus and transcribing genes, including Ptch1 for negative feedback and Gli1 for positive feedback.[410] Noncanonical signaling is independent of Gli and can be further subdivided into two types, type I which modulates Ca^{2+} and the actin cytoskeleton via downstream actions of Smo, and type II which increases cell proliferation and survival and is Smo-independent.[410]

Hh signaling can recruit CK1 to phosphorylate Smo to increase its cell-surface accumulation, leading to stronger Hh signaling.[411,412] CK1 can also stabilize Gli by phosphorylating Ser/Thr-rich motifs in the N- and C-terminal regions of Gli to prevent degradation by HIB.[413] Additionally, CK1 can phosphorylate and activate the Fused (Fu) protein kinase, a kinase that phosphorylates Gli for activation.[414] Further regulation of Hh signaling can be induced by GSK-3β, which can phosphorylate suppressor of fused (SUFU), which normally binds the Gli transcription factors and prevents them from entering the nucleus. Phosphorylation of SUFU by GSK-3β prevents SUFU from binding to the Gli transcription factors, presenting a positive regulatory role for GSK-3β in Hh signaling.[415] One of the downstream genes that are transcribed by the Hh signaling cascade is sFRP1. Specifically, Gli1 and Gli2 are responsible for the transcription. In other words, the activation of Hh signaling inhibits Wnt signaling via the production of sFRP1, indicating a regulatory role of the Hh pathway for the Wnt pathway.[416] Furthermore, SUFU can also decrease β-catenin levels via nuclear export and in turn down-regulate TCF-dependent transcription.[417]

Crosstalk with fibroblast growth factor (FGF) signaling

Fibroblast growth factors (FGFs) are a family of 22 polypeptide growth factors in seven subfamilies that are involved in diverse cellular processes. The binding of FGFs to FGF receptors (FGFRs) causes receptor dimerization that is promoted by heparan sulfate. This leads to autophosphorylation of FGFR that can activate the MAPK, phosphatidylinositol-3 kinase/Akt (PI3K/Akt), and phospholipase C-γ (PLC-γ) pathways.[418] They also have interactions with the Wnt signaling pathway. Akt from the PI3K/Akt pathway phosphorylates Ser9 on GSK-3β, decreasing its activity. This reduces the ability of GSK-3β to phosphorylate SNAIL for degradation. SNAIL usually represses transcription of the E-cadherin gene, CDH1, which maintains cell adhesion. In this manner, FGF signaling can stimulate the EMT via decreasing E-cadherin levels by GSK-3β inhibition.[419] Activation of the PI3K/Akt pathway by FGF also releases β-catenin from a complex composed of E-cadherin, β-catenin, and α-catenin, enabling β-catenin to translocate to the nucleus.[419] FGF18 and FGF20 are target genes of the canonical Wnt pathway, indicating that Wnt signaling can mediate FGF signaling activation.[419] In the development of the anterior heart field (AHF) in mice, Wnt/β-catenin signaling regulates the expression of FGF3, 10, 16, and 20 in AHF progenitor cells.[420] Mesenchymal FGF signaling is vital in Wnt2a expression stabilizing β-catenin, and Wnt/β-catenin signaling sustains expression of FGFR1c/2c and responsiveness to FGF9, creating a positive feedback loop for mesenchymal proliferation in the lung.[421]

Crosstalk with parathyroid hormone (PTH) signaling

Parathyroid hormone (PTH) is a protein implicated in bone remodeling and acts through two G-protein coupled pathways, the Gs/cAMP/protein kinase A (PKA) pathway and the Gq/PLC/Ca^{2+}/protein kinase C (PKC) pathway.[422] The bone repair effects of PTH are partly mediated by Wnt signaling, as PTH-treated bones had increased numbers of β-catenin-expressing osteoblastic cells in the fracture callus. These fracture calluses also demonstrated increased levels of Wnt4, 5a, 5b, 10, and 11.[423] PTH also enhances the transcriptional activity induced by β-catenin by promoting the expression of Smad3.[424] Additionally, PTH appears to be able to stimulate Wnt/β-catenin signaling to prevent

apoptosis, but this does not require PTH, suggesting the need for further studies to clarify this role.[424] PTH can stimulate osteoclastogenesis in an opposing manner to Wnt signaling, which inhibits osteoclastogenesis. PTH and Wnt signaling modulates β-catenin in different manners to cause these opposite effects.[425] The PTH receptor (PTH1R) can recruit Dvl and activate β-catenin signaling without Wnt or LRP5/6. This association of PTH1R and Dvl is what enables PTH-induced osteoclastogenesis.[425]

Crosstalk with other pathways

Wnt signaling can interact with multiple other pathways in addition to the BMP, Notch, and Hippo signaling pathways. Analysis of mouse hepatocytes found that high insulin levels can stimulate the Wnt/β-catenin pathway to promote lipogenic gene expression. Insulin activates stearoyl-CoA-desaturase 1 (SCD1), an enzyme involved in lipogenesis that supplies palmitoleate for Porc, enabling it to acylate Wnt ligands for secretion.[426] Insulin can also enhance Wnt signaling by activating Akt to phosphorylate and inactivate GSK-3β, which leads to increased glycogen storage, glucose clearance, and insulin sensitivity. As such, GSK-3β inhibitors have become a therapeutic target in the treatment of type II diabetes.[318]

PP2A is another component of Wnt signaling that is involved in other pathways. For instance, it is involved in the mechanistic target of the rapamycin (mTOR) pathway, which is vital in cell growth and metabolism.[342] PP2A can negatively regulate the mTOR signaling pathway by dephosphorylating and inactivating Akt, which is normally activated by mTOR. PP2A can also inhibit IRS1, which is upstream of Akt. However, mTOR can also inhibit PP2A, suggesting a complex interaction between Wnt signaling and mTOR signaling.[342] PP2A also has a major role in both positively and negatively regulating the MAPK pathway. In terms of negative regulation, PP2A can bind the phosphotyrosine-binding (PTB) domain of Shc, an adaptor protein that acts as a signal transducer in the Ras/MAPK pathway. PP2A can inhibit MAPK activation, but this association of PP2A to Shc can be alleviated by growth factor stimulation or small-t antigen expression.[427] For positive regulation, the PR130 regulatory subunit of PP2A can form a holoenzyme that complexes with SRC homology 2 domain-containing inositol polyphosphate phosphatase (SHIP2). This complex stabilizes the EGF receptors (EGFR) that are necessary for the ERK/MAPK signaling pathway.[428]

Conclusions and future directions

The study of Wnt signaling has progressively provided more insight into this complex pathway. The structures and many interactions of the Wnt signaling components have been established, providing a breadth of knowledge about the crosstalk capability of Wnt signaling with a heavy emphasis on its vast effects in different organs. The conservation of the Wnt ligands, Fz receptors, and coreceptors, has further driven home the fundamental importance of the Wnt signaling pathway. Additionally, the antagonists of Wnt signaling also have functions beyond just the Wnt pathway, adding further complexity to the interactions of Wnt signaling with other pathways.

However, many details are still needed to be clarified. For the 19 Wnt ligands, it is not completely clear how each ligand interacts with Fz receptors and LRP5/6 coreceptors. The specificity of the interaction between these receptors and the Wnt ligands needs to be further clarified. Further research continues to uncover more and more pathways with which Wnt signaling interacts, yet the number of pathways and proteins that crosstalk with Wnt signaling is still not fully determined. Furthermore, the mechanisms of many Wnt signaling interactions with other pathways are not fully elucidated. The functions of each component of the Wnt signaling pathway are diverse and may still not be completely known yet.

Due to the implication of dysregulated Wnt signaling in diseases such as cancer, therapeutics have been developed. However, like the Wnt signaling pathway and components, the therapeutics also require caution in their function and use. Both activators and inhibitors of the Wnt signaling pathway can come with their own risk of tumorigenesis or unintentional inhibition of important cellular functions, which can lead to organ dysfunction. Furthermore, if an activator or inhibitor is affected by treatment, it may be unknown what potential effects can occur if that activator or inhibitor is also involved in another pathway. Additional research is needed to further clarify the therapeutic effects of various treatment options, as well as ensure their safety.

Although the current analysis of Wnt signaling is not completely exhaustive, the broad amount of research on the Wnt signaling pathway has provided us with significant insight into the system and its many functions in living organisms over the last 40 years. Hopefully, in the future, more research will be performed to provide more knowledge on the Wnt signaling pathway, leading to further advancements in biology, medicine, and human health.

Conflict of interests

Tong-Chuan He is the Editor-in-Chief of *Genes & Diseases*. To minimize bias, he was excluded from all editorial decision-making related to the acceptance of this article for publication. The remaining authors declare no conflict of interests.

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