Protein family review

The Wnts

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Summary

The *Wnt* genes encode a large family of secreted protein growth factors that have been identified in animals from hydra to humans. In humans, 19 WNT proteins have been identified that share 27% to 83% amino-acid sequence identity and a conserved pattern of 23 or 24 cysteine residues. *Wnt* genes are highly conserved between vertebrate species sharing overall sequence identity and gene structure, and are slightly less conserved between vertebrates and invertebrates. During development, Wnts have diverse roles in governing cell fate, proliferation, migration, polarity, and death. In adults, Wnts function in homeostasis, and inappropriate activation of the Wnt pathway is implicated in a variety of cancers.

Gene organization and evolutionary history

Gene organization

In humans, 19 *WNT* genes have been identified and the chromosomal locations of each is known (see Table 1) [1-6]. Several human *WNT* genes are located very close to each other in the genome [7,8]; these include *WNT6* and *WNT10a*, which are located immediately adjacent to one another on chromosome 2 (about 6.4 kilobases (kb) apart), and *WNT1* and *WNT10b*, which are located adjacent to each other on chromosome 12 (about 8.1 kb apart). *WNT6* and *WNT10a* are transcribed in opposite directions, whereas *WNT1* and *WNT10b* are expressed from the same strand of DNA. Several additional pairs of *WNT* genes are also clustered within the human genome, including *WNT2* and *WNT16* (about 4 megabases (Mb) apart), *WNT3a* and *WNT14* (about 250 kb apart), and *WNT3* and *WNT15*. In the mouse, there are at least 18 *Wnt* genes and the locations of all but two of them have been determined [1-3,5,6]. As in humans, the mouse *Wnt1*/*Wnt10b*, *Wnt6*/*Wnt10a*, and *Wnt3*/*Wnt15* gene pairs are each located on the same chromosomes, and in the case of the *Wnt1*/*Wnt10b* and *Wnt6*/*Wnt10a* pairs the close proximity of these genes has been conserved from mouse to human. Interestingly, in the *Drosophila* genome, the paralogous genes *wingless* (*wg*), *DWnt6* and *DWnt10*, are located immediately adjacent to one another on the second chromosome and are all transcribed in the same orientation. Thus, it is possible that there was an ancient cluster of *Wnt* genes consisting of *Wnt1*, *Wnt6* and *Wnt10* in a common ancestor of vertebrates and arthropods. In vertebrates, this cluster may have been duplicated with subsequent loss of *Wnt1* from one cluster and *Wnt6* from the other.

The majority of human *WNT* genes contain four coding exons, with exon 1 containing the initiation methionine (Figure 1a) [8]. *WNT* genes that differ from this pattern include *WNT14*, with three exons, *WNT2*, *WNT5b*, and *WNT11*, with five exons, and *WNT8b* with six exons. Several *WNTs* - *WNT2b/13*, *WNT8a/d*, and *WNT16* - have alternative amino or carboxyl termini, which result from the use of alternative 5′ or 3′ exons.

Evolutionary history

The deduced evolutionary relationships of 18 of the 19 known human *WNT* genes are shown in Figure 2. The majority of Wnt proteins share about 35% amino-acid sequence identity, although members of a subgroup (those with the same numeral, such as *WNT3* and *WNT3a*) share increased sequence identity (from 58% to 83%) and some overlapping sites of expression. Members of subgroups are not closely linked within the genome, however, suggesting that they were generated by gene-translocation or genome-duplication events, not by local duplication events.

Table 1

Chromosomal locations of *WNT* genes in human and mouse

Human		Mouse		References	Accession numbers†	
Gene	Location	Gene	Location*		Human	Mouse
WNT1	12q13	*Wnt1*	15	[87-91]	X03072	K02593
WNT2	7q31	*Wnt2*	6 (4.2 cM)	[92,93]	X07876	AK012093
WNT2b/13	1p13	*Wnt2b/13*	3 (49.0 cM)	[94-96]	XM052111, XM052112	AF070988
WNT3	17q21	*Wnt3*	11 (63.0 cM)	[97-100]	AY009397	M32502
WNT3a	1q42.13	*Wnt3a*	11 (32.0 cM)	[101-103]	AB060284	X56842
WNT4	1p35	*Wnt4*	4	[100,104]	AY009398	M89797
WNT5a	3p14-p21	*Wnt5a*	14 (14.8 cM)	[104-106]	L20861	M89798
WNT5b	12p13.3	*Wnt5b*	6 (56.2 cM)	[104,107]	AB060966	M89799
WNT6	2q35	*Wnt6*	1	[104,108,109]	AY009401	M89800
WNT7a	3p25	*Wnt7a*	6 (39.5 cM)	[104,106,110,111]	D83175	M89801
WNT7b	22q13.3	*Wnt7b*	15 (46.9 cM)	[100,104,112,113]	AB062766	M89802
WNT8a/d	5q31	*Wnt8a*		[114,115]	AB057725, AY009402	Z68889
WNT8b	10q24	*Wnt8b*	19 (43.0 cM)	[116-118]	Y11094	AF130349
WNT10a	2q35	*Wnt10a*	1	[109,119]	AB059569	U61969
WNT10b/12	12q13.1	*Wnt10b*	15 (56.8 cM)	[106,119-124]	U81787	U61970
WNT11	11q13.5	*Wnt11*	7	[106,125]	Y12692	X70800
WNT14	1q42	-		[103,126]	AB060283	
WNT15	17q21	*Wnt15*	11	[126]	AF028703	AF031169
WNT16	7q31	*Wnt16*		[127,128]	XM031374, XM004884	AF172064

*Locations of mouse genes give the chromosome and the distance in centimorgans (cM) from the telomere. †Accession numbers are for GenBank [3].

Wnt genes have been identified in vertebrates and invertebrates, but appear to be absent from plants, unicellular eukaryotes such as *Saccharomyces cerevisiae* and from prokaryotes. To date, in vertebrates, 16 *Wnt* genes have been identified in *Xenopus*, 11 in chick, and 12 in zebrafish [5]; in invertebrates, *Drosophila* has seven *Wnt* genes, *Caenorhabditis elegans* five and *Hydra* at least one [5]. The apparent evolutionary relationships between selected invertebrate and vertebrate *Wnt* genes are shown in Figure 2b. In vertebrates, the orthologs in different species are highly similar in sequence. For example, human WNT1 and mouse Wnt1 are 98% identical, and human WNT5a and *Xenopus* Wnt5a are 84% identical at the amino-acid level. Phylogenetic analyses of vertebrate and invertebrate Wnts demonstrate orthologous relationships between several human and *Drosophila Wnts* (Figure 2b). The sequence identity between orthologous proteins in humans and flies ranges from 21% between human WNT8a/d and *Drosophila* DWnt8 to 42% sequence identity between human WNT1 and *Drosophila* Wingless (Wg). The evolutionary relationship between the five *C. elegans Wnt* genes and human *WNT* genes is less apparent, making it difficult to determine which *C. elegans Wnt* genes may have orthologs in the human genome.

Characteristic structural features

Human WNT proteins are all very similar in size, ranging in molecular weight from 39 kDa (WNT7a) to 46 kDa (WNT10a) [3]. *Drosophila* Wnt proteins are also similar to this, with the exception of Wg, which is approximately 54 kDa and has an internal insert not found in vertebrate Wnts, and DWnt3/5, which is about 112 kDa [3]. Very little is known about the structure of Wnt proteins, as they are notoriously insoluble, but all have 23 or 24 cysteine residues, the spacing of which is highly conserved (Figure 1b), suggesting that Wnt protein folding may depend on the formation of multiple intramolecular disulfide bonds. Analysis of the signaling activities of chimeric Wnt proteins has shown that the carboxy-terminal region of Wnt proteins may play a role in determining the specificity of responses to different Wnts [9]. Furthermore, deletion mutants lacking the carboxy-terminal third of a Wnt protein can act as

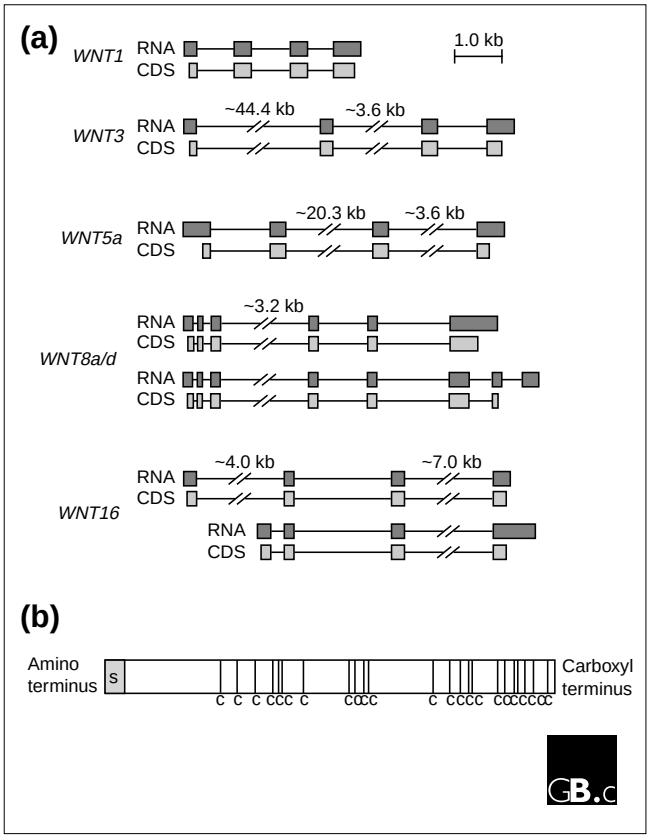

Figure 1
(a) Structures of selected members of the human *WNT* gene family. Exons are shown as boxes and introns as lines. For each gene, 'RNA' represents the portion of the gene that is transcribed and 'CDS' represents the portion that encodes protein. *WNT8a/d* is an example of a gene with 3′ alternative splicing and *WNT16* is an example of a gene with alternatively used 5′ exons. **(b)** Structural features of the Wnt protein. The amino terminus contains a signal sequence (S). All Wnts contain 23 or 24 conserved cysteine residues (C) with similar spacing, suggesting that the folding of Wnt proteins depends on the formation of multiple intramolecular disulfide bonds.

dominant-negatives in a cell-non-autonomous manner [10], suggesting that the amino-terminal region may mediate interactions with Wnt receptors but requires the carboxyl terminus to activate these receptors.

Localization and function

Post-translational modifications and secretion

Wnt proteins have an amino-terminal signal sequence, can act in a cell non-autonomous manner, and are present in the secretory pathway, indicating that they are secreted proteins [11]. In addition, genetic analyses of Wg signaling in *Drosophila* uncovered mutations in the *porcupine* gene that show a lack of Wnt activity due to the retention of Wg protein in the endoplasmic reticulum [12-14]. The *porcupine* gene is predicted to encode a protein with eight transmembrane domains and has a perinuclear localization in transfected cells [14]; overexpression of *porcupine* does not increase levels of secreted Wg but does change the pattern of Wg glycosylation [14]. In worms, *mom-1* encodes a *porcupine* homolog and, when mutated, phenocopies mutants of *mom-2*, which encodes a Wnt, suggesting that the function of *porcupine* is conserved [15,16]. Although size chromatography suggests that Wg is secreted as a multimer, it remains unclear whether Wnt proteins in general are secreted as monomers, oligomers, or as part of a multi-protein complex [17]. Wnt proteins are glycosylated, but mutation of some or all of the predicted glycosylation sites in mouse Wnt1 does not abolish its activity in cultured cells [18]; these modifications may thus be unimportant for Wnt function.

Subcellular localization

Once secreted, Wnt proteins associate with glycosaminoglycans in the extracellular matrix and are bound tightly to the cell surface [19,20]. Although Wnts are found in tight association with the plasma membrane, it is possible to collect active Wnt from the medium of cultured cells [21,22]. Beyond this information, the localization of Wnt proteins in vertebrates is poorly understood. Examination of the localization of Wg in *Drosophila*, however, has provided critical insights into the subcellular distribution of Wnt proteins and the importance of this distribution for signaling activity. In the embryonic epidermis, Wg is found inside cells that secrete Wg and in association with the plasma membrane of secreting cells and non-secreting cells several cell diameters from the Wg source [23]. Wg is also prevalent in vesicles and multi-vesicular bodies of non-Wg-producing cells anterior to the source of Wg, suggesting that Wg is endocytosed [23,24]. This idea is supported by examination of *shibire* embryos, which have a mutation in dynamin, a critical component of the endocytic machinery; these mutants have defects in Wg distribution, and Wg signaling activity is compromised [25]. Similarly, expression of a dominant-negative form of *shibire* also reduces Wg activity [26]. Endocytosis may also help to limit the distribution of Wg signal. In contrast to cells anterior to the Wg source, cells posterior to Wg-producing cells have much lower levels of Wg in endocytic vesicles, and this asymmetry in distribution mirrors the observation that Wg acts over a much shorter range towards the posterior than towards the anterior. This difference in Wg distribution appears to be due to rapid degradation of endocytosed Wg in posterior cells [27]. The spatially restricted pattern of Wg degradation is regulated by signals through the epidermal growth factor (EGF) receptor that hasten the destruction of Wg in posterior cells [27].

Association of Wg with specific membrane microdomains also appears to play a role in controlling the distribution of Wg signals during *Drosophila* development. In imaginal discs, Wg is found in specialized membrane vesicles called argosomes, which are thought to be derived from lipid raft microdomains [28]. Incorporation of Wg into argosomes requires heparan sulfate proteoglycans, suggesting that

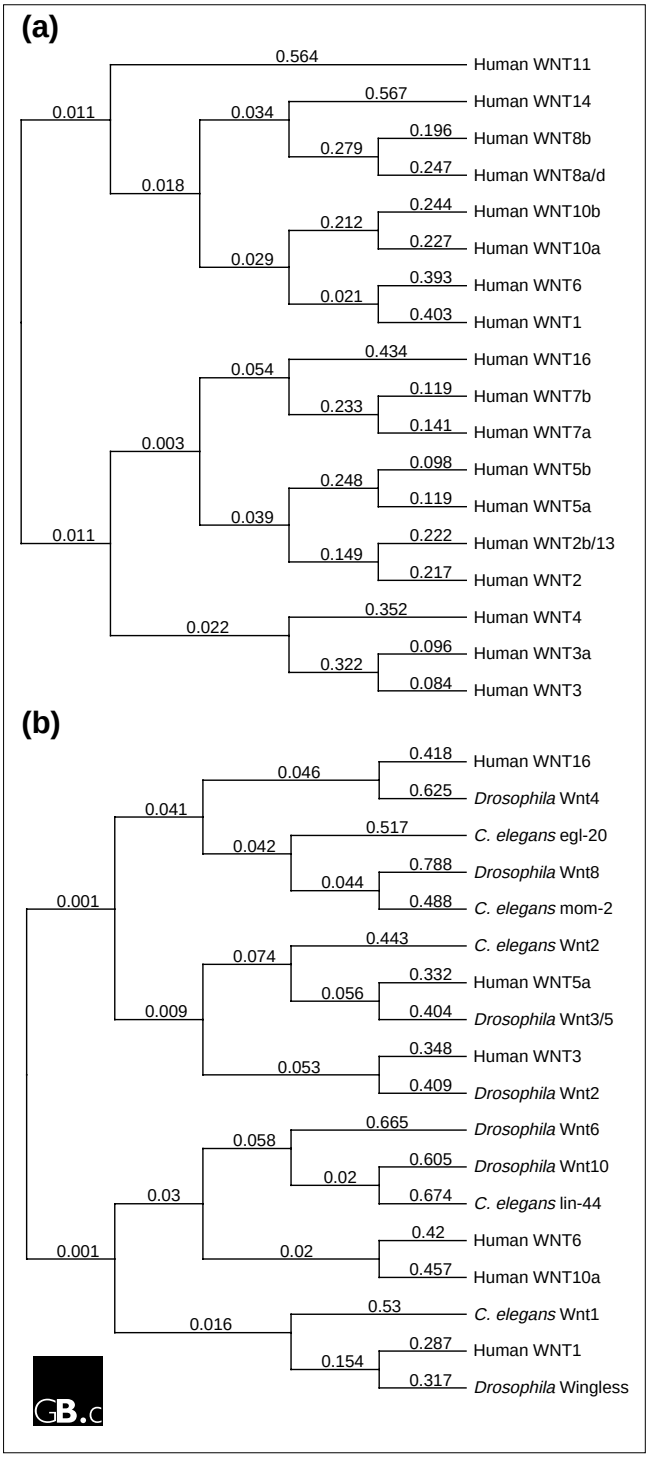

Figure 2
Predicted evolutionary relationships between members of the *Wnt* gene family. **(a)** Predicted relationships between 18 of the 19 known human *WNT* protein sequences; *WNT15* was omitted because only a partial sequence is available. **(b)** Predicted evolutionary relationships between selected human *WNT* proteins (representing each large grouping shown in (a)) and *Wnt* proteins from mouse, *Xenopus, Drosophila,* and *Caenorhabditis elegans.* Sequences were aligned using the ClustalW program; trees were constructed from the alignments using the neighbor-joining method and are diagrammed using midpoint rooting. Numbers indicate branch lengths.

proteoglycans play a role in sorting Wg to specialized membrane microdomains in Wg-producing cells or, alternatively, may play a role localizing Wg in distinct endocytic compartments in receiving cells.

Polarized distribution of *wg* transcripts in embryonic epithelial cells is also required for optimal signaling activity. High-resolution *in situ* hybridization analyses demonstrate that *wg* transcripts are localized apically in the embryonic epidermis and that this distribution is mediated by two *cis*-acting elements found in the 3′ UTR of the *wg* mRNA [29]. Mutation of these elements results in uniform localization of *wg* transcripts and impaired Wg protein distribution and signaling. The asymmetric distribution of *wg* transcripts is dependent on dynein-mediated microtubule transport [30].

Function

Wnts and Wnt receptors

Reception and transduction of Wnt signals involves binding of Wnt proteins to members of two distinct families of cell-surface receptors, members of the Frizzled (Fzd) gene family and members of the LDL-receptor-related protein (LRP) family [31,32]. The canonical Fzd receptor has an amino-terminal cysteine-rich domain (CRD) that binds Wnt, seven transmembrane domains and a short cytoplasmic tail containing a consensus PDZ domain binding motif (S/T-X-V in the single-letter amino-acid code) at the carboxyl terminus. The CRD forms a novel protein fold with a conserved dimerization interface that may be important for Wnt binding [33]. Fzd receptors have been identified in vertebrates and invertebrates; there are ten known members in humans and mice, four in flies, and three in worms. The general structure of Fzd receptors resembles that of seven-transmembrane G-protein-coupled receptors, suggesting that Fzd proteins may use heterotrimeric G proteins to transduce Wnt signals. Several recent studies provide evidence consistent with this idea, showing that a subgroup of Fzd receptors can signal through the pertussis-toxin-sensitive subclass of heterotrimeric G proteins to stimulate an increase in intracellular Ca^{2+} and activate protein kinase C (PKC) [34-38]. Heterotrimeric G proteins do not appear to be involved in transducing Wnt/Fzd signals that regulate the cytoskeleton-associated protein β-catenin, however (see below).

Two members of the vertebrate LRP family, LRP-5 and LRP-6, can bind Wnts and may form a ternary complex with a Wnt and a Fzd [39]. Mutations in *LRP-6* in mice result in developmental defects similar to those seen in mice deficient for several individual *Wnt* genes [40], and overexpression of LRP in *Xenopus* can activate the Wnt pathway [39]. In *Drosophila*, *arrow,* the ortholog of LRP5 and LRP6, is required for optimal Wg signaling [41]. Although the mechanism of LRP signaling is unclear, recent evidence suggests that binding of the cytoplasmic domain of LRP to the Wnt antagonist Axin may play a role in Wnt pathway activation [42].

In addition to the Fzd and LRP receptors, cell-surface proteoglycans also appear to have a role in the reception of Wnt signals. For example, genetic analyses in *Drosophila* have shown that several genes required for optimal Wg signaling encode cell-surface proteoglycans of the glypican family [43,44] and proteins involved in proteoglycan synthesis [45-47]. Furthermore, QSulf1, an avian protein related to heparan-specific *N*-acetyl glucosamine sulfatases, has also been shown to regulate heparan-dependent Wnt signaling in cultured cells [48]. It is unclear at this time how proteoglycans modulate Wnt signaling, but current suggestions include concentrating Wnt proteins at the cell surface or presenting Wnt ligands to cell-surface receptors.

Secreted modulators of Wnt signaling

Wnt signals are modulated extracellularly by diverse secreted proteins, including members of the Frizzled-related protein (FRP or FrzB) family [49], Wnt-inhibitory factor-1 (WIF-1) [50], Cerberus [51], and Dickkopf (Dkk) [52]. FRPs, WIF-1, and Cerberus can bind Wnt proteins directly and are thought to antagonize Wnt function by preventing their interaction with Fzd receptors. FRPs can also interact with Fzds, suggesting that a second way in which FRPs might antagonize Wnt signaling is through the formation of a nonfunctional complex with Fzd receptors. Humans have at least five *FRP* genes, and the specificity of each FRP for different Wnts remains to be determined. Dkk does not bind Wnts but instead interacts with the extracellular domain of LRPs, thereby blocking activation of Wnt signaling [42,53,54]. Four *Dkk* genes have been identified in vertebrates, including *Dkk2*, which does not act as a Wnt antagonist but rather can stimulate Wnt signaling [55].

Intracellular signaling pathways

Wnt signals are transduced through at least three distinct intracellular signaling pathways including the canonical 'Wnt/β-catenin' pathway, the 'Wnt/Ca^{2+}' pathway, and the 'Wnt/polarity' pathway (also called the 'planar polarity' pathway) [5,56-62]. Distinct sets of Wnt and Fzd ligand-receptor pairs can activate each of these pathways and lead to unique cellular responses. The Wnt/β-catenin pathway primarily regulates cell fate determination during development, whereas the major function of the Wnt/polarity pathway is regulation of cytoskeletal organization. The biological function of the Wnt/Ca^{2+} pathway is unclear.

The canonical Wnt/β-catenin pathway is intensely studied, and on the basis of current literature I propose the model illustrated in Figure 3a [59,63,64]. Signaling through this pathway depends on the levels of β-catenin in the cell. In the absence of Wnt, β-catenin is targeted for degradation by a multi-protein destruction complex. Wnt signaling antagonizes the destruction complex, leading to the accumulation of β-catenin and activation of target genes. Up-to-date lists of proteins involved in Wnt/β-catenin signaling and the potential roles of each of these proteins can be found on the worldwide web [5,60,62].

The Wnt/Ca^{2+} pathway involves an increase in intracellular Ca^{2+} and activation of PKC; it can be activated by a distinct group of Wnt ligands and Fzd receptors from those that activate other pathways, including Wnt5a, Wnt11 and Fzd2 (Figure 3b) [58,61,62]. The Wnt/Ca^{2+} pathway involves activation of a heterotrimeric G protein, an increase in intracellular Ca^{2+}, and activation of calcium/calmodulin-regulated kinase II (CamKII) and PKC [34,35,37]. The downstream targets of CamKII and PKC are currently unknown, but it has been shown that activation of the Wnt/Ca^{2+} pathway can antagonize the Wnt/β-catenin pathway in *Xenopus*, although it is unclear at what level this interaction occurs [65].

Wnt/polarity signaling regulates the polarity of cells through regulation of their cytoskeletal organization (Figure 3c) [56,57,62]. In vertebrates, Wnt/polarity signaling is thought to control polarized cell movements during gastrulation and neurulation [66-70]. In *Drosophila*, Wnt/polarity signaling

Figure 3 *(see the figure on the next page)*
The known Wnt signaling pathways. **(a)** In the Wnt/β-catenin pathway, Wnt signaling depends on the steady-state levels of the multi-functional protein β-catenin. In the absence of Wnt signal, a multi-protein destruction complex that includes the adenomatous polyposis coli protein (APC) and a member of the Axin family facilitates the phosphorylation of β-catenin by glycogen synthase kinase 3 (GSK3). GSK3 substrates also include APC and Axin; phosphorylation of each of these proteins leads to enhanced binding of β-catenin. Phosphorylated β-catenin is bound by the F-box protein β-TrCP, a component of an E3 ubiquitin ligase complex, and is ubiquitinated; the ubiquitin tag marks β-catenin for destruction by the proteasome. When a cell is exposed to a Wnt, the Wnt interacts with its coreceptors Frizzled and LRP. Activation of Frizzled and LRP leads to the phosphorylation of Dishevelled (Dsh), a cytoplasmic scaffold protein, perhaps through stimulation of casein kinase Iε (CKIε) and/or casein kinase II (CKII). Dsh then functions through its interaction with Axin to antagonize GSK3, preventing the phosphorylation and ubiquitination of β-catenin. In vertebrates, inhibition of GSK3 may involve the activity of GSK3 binding protein (GBP/Frat), which binds to both Dsh and GSK3 and can promote dissociation of GSK3 from the destruction complex. Unphosphorylated β-catenin escapes degradation, accumulates in the cell, and enters the nucleus, where it interacts with members of the TCF/LEF family of HMG-domain transcription factors to stimulate expression of target genes. In addition to the components of the Wnt/β-catenin pathway described here, many additional proteins with potential roles in regulating Wnt/β-catenin signaling have been reported including the phosphatase PP2A and the kinases Akt/protein kinase B, integrin-linked kinase (ILK), and PKC. **(b)** Signaling through the Wnt/Ca^{2+} pathway appears to involve activation of the two pertussis-toxin-sensitive G proteins, $G_{\alpha o}$ and $G_{\alpha t}$, in combination with $G_{\beta 2}$ [34,35]. G-protein activation then leads to an increase in intracellular Ca^{2+} and the subsequent stimulation of Ca^{2+}/calmodulin-dependent kinase II (CamKII) [37]. Activation of the Wnt/Ca^{2+} pathway also results in stimulation of PKC activity in the form of the translocation of PKC to the plasma membrane [34]. Downstream targets of the Wnt/$Ca2^{+}$ pathway have not been identified. **(c)** The Wnt/polarity pathway, which regulates cytoskeletal organization; the *Drosophila* Wnt/polarity pathway that regulates the polarity of trichomes in the wing is shown as an example. In this case, the nature of the polarity signal is not known.

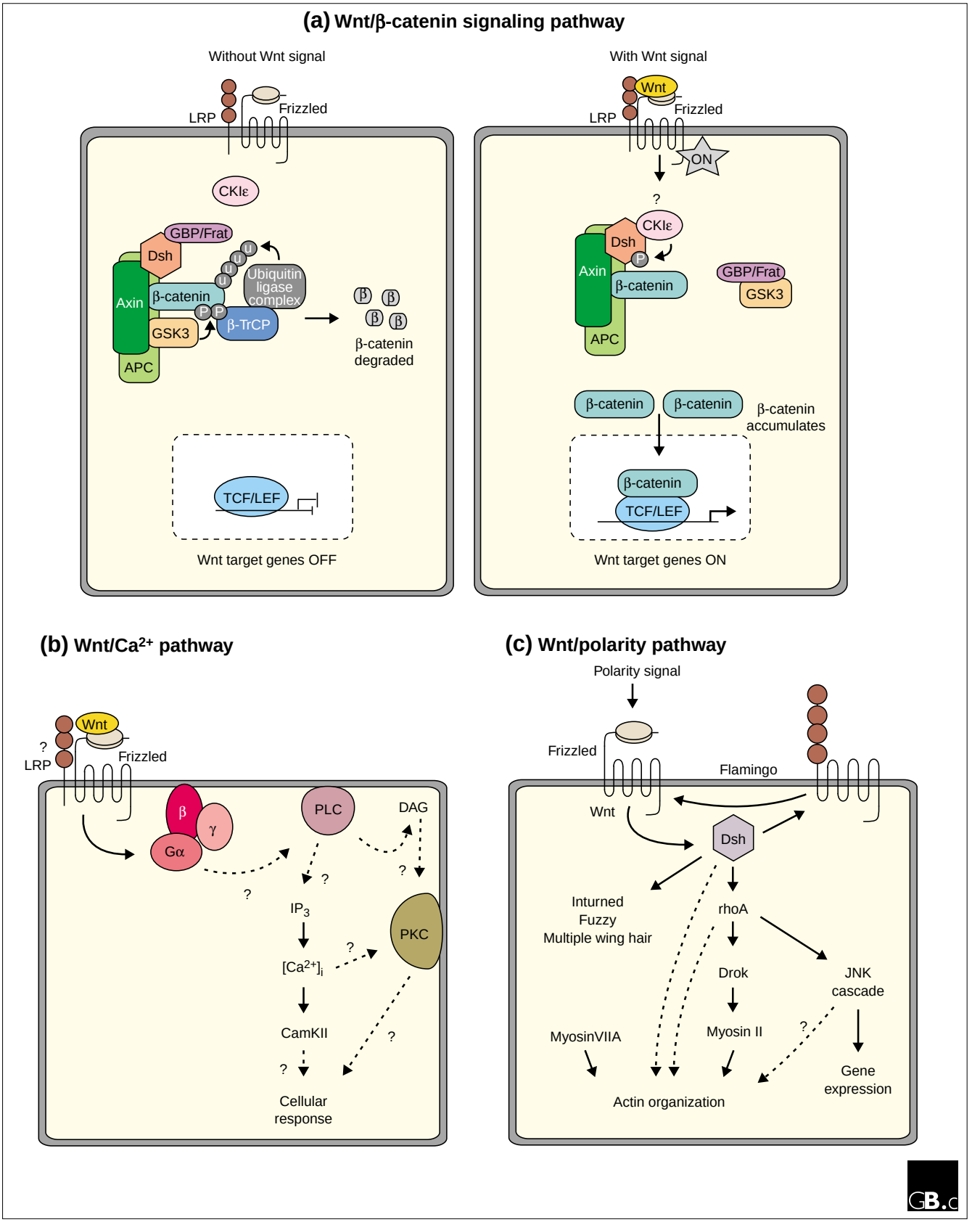

Figure 3 *(see the legend on the previous page)*

is required for the appropriate orientation of trichomes - or hairs - of the adult wing and for appropriate chirality of ommatidia in the eye, and may regulate asymmetric cell divisions of certain neuroblasts [56,71,72]. The only molecules known to function in both the vertebrate and the invertebrate Wnt/polarity pathways are members of the Fzd family and the cytoplasmic scaffold protein Dsh. The regulation of gastrulation movements in vertebrates also requires the activity of *Wnt11*, which may signal through Fzd7 to regulate protrusive activity during convergent extension [66,67]. In flies, genetic analyses have identified a number of potential components of the Wnt/polarity pathway in addition to DFzd1 and Dsh, including the small GTPase DrhoA, *Drosophila* rho-associated kinase (Drok), Jun N-terminal kinase (JNK), myosin II, myosin VIIA, and the products of the novel genes *flamingo/starry night*, *fuzzy*, *inturned*, and *strabismus/van gogh* [56,72]. A Wnt ligand for the Wnt/polarity pathway has not been identified in flies, however, and it remains to be seen how much of the intracellular signaling mechanism has been conserved between vertebrates and invertebrates.

Several studies have suggested that distinct classes of Wnts signal through either the Wnt/β-catenin pathway or the Wnt/Ca^{2+} pathway [58]; for example, overexpression studies in *Xenopus* have shown that XWnt1, XWnt3a, XWnt8, and XWnt8b can stimulate the Wnt/β-catenin pathway whereas XWnt4, XWnt5a, and XWnt11 can stimulate the Wnt/Ca^{2+} pathway [58]. Furthermore, the separation of Wnts into these two distinct functional classes is mirrored by the classification of Fzd proteins into similar functional groups on the basis of their ability to activate one or other pathway in overexpression assays. Although this classification of Wnts, which partially mirrors their evolutionary relationships, may provide a useful tool for predicting the function of Wnts and Fzds, the relationship between specific Wnts and the intracellular pathway they use is not fixed. For example, overexpression of XWnt5a in combination with human FZD5 in *Xenopus* embryos results in activation of the Wnt/β-catenin pathway [73], suggesting that the activity of Wnts *in vivo* will be determined by the repertoire of Fzd receptors present at the cell surface.

Important mutants and developmental functions

Loss-of-function mutations in 9 of the 18 mouse *Wnt* genes have been generated, and the phenotypes of mutant embryos demonstrate the diverse functions of *Wnt* genes during embryogenesis (Table 2). For example, knocking out *Wnt1* results in a dramatic loss of a portion of the midbrain and deletion of the rostral cerebellum [74,75]. Inactivation of *Wnt4* results in the absence of kidneys [76], masculinization of mutant females (absence of the Müllerian duct and continued development of the Wolffian duct) [77], and defects in mammary gland morphogenesis during pregnancy [78]. Targeted knockout of *Wnt7a* also has pleiotropic effects, including ventralization of the limbs

Table 2

Developmental functions of mouse *Wnt* genes

Gene	Natural allele	Phenotype of knockout or other functions	References
Wnt1	*swaying*	Loss of a portion of the midbrain and cerebellum	[74,75,129,130]
		Deficiency in dorsal neural-tube derivatives, including neural-crest cells in double knockout with *Wnt3a*	[131]
Wnt2		Placental defects	[132]
Wnt3		Defects in axis formation and gastrulation	[84]
		Defects in hair growth and structure	[133,134]
Wnt3a	*vestigial tail*	Defects in somite and tailbud development	[102,135-137]
		Deficiency in dorsal neural-tube derivatives, including neural crest cells in double knockout with *Wnt1*	[131]
		Loss of hippocampus	[138]
Wnt4		Defects in kidney development	[76]
		Defects in female development; absence of Müllerian duct, ectopic synthesis of testosterone in females	[77]
		Defects in mammary gland morphogenesis	[78]
Wnt5a		Truncated limbs, shortened anterior-posterior axis, reduced number of proliferating cells	[139]
Wnt7a	*postaxial hemimelia*	Defects in limb polarity	[79]
		Female infertility due to failure of Müllerian duct regression	[80,140]
		Defects in uterine patterning	[141]
		Defects in synapse maturation in the cerebellum	[81]
Wnt7b		Placental defects	[142]
Wnt10b		Inhibition of adipogenesis	[143]

[79], female infertility due to failure of Müllerian-duct regression [80], and a delay in the morphological maturation of glomerular rosettes in the cerebellum [81].

Overexpression and antisense 'knockdown' analyses in *Xenopus* have shown that the Wnt/β-catenin pathway is required for the specification of dorsal cell fates [82]. A debate is ongoing, however, over whether a maternal Wnt ligand is required to activate this pathway in dorsal cells. In support of a role for a Wnt ligand, a recent study has shown that *XFzd7* is important for establishing dorsal cell fates [83], thereby implicating a Wnt ligand in this process. Furthermore, targeted knockout of *Wnt3* in mice results in defects in axis formation and gastrulation, suggesting a conserved role for Wnts in regulating the establishment of the dorsal-ventral axis in vertebrates [84]. On the other hand, overexpression of a dominant-negative form of *Xwnt8* in oocytes does not suppress formation of dorsal cell fates, arguing against the requirement for a maternal Wnt in axis specification [10]. Further studies are necessary to resolve the role of Wnts in vertebrate early axial development.

In flies, Wnt signaling has a variety of functions during development. The *wg* gene is required for cell-fate choices in the ventral epidermis during embryogenesis, as well as for many other functions, and *DWnt2* is required for testis and adult muscle development [17]. In *C. elegans*, genetic analyses have defined a number of roles for Wnts, including establishment of polarity and endodermal cell fates in the early embryo and regulation of cell migration, among many others [85]. A comprehensive list of *Wnt* genes and their mutant phenotypes in vertebrates and invertebrates can be found at the *Wnt* gene homepage [5].

Wnt signaling and cancer

In addition to the many roles for Wnt signaling during development and in adult tissues, it is also involved in tumorigenesis in humans [59,64]. Although mutation or misexpression of a *Wnt* gene has not been linked directly to cancer in humans, mutation of several intracellular components of the Wnt/β-catenin pathway is thought to be critical in many forms of cancer. Most notably, patients with familial adenomatous polyposis (FAP) develop multiple intestinal adenomas early in life and have germline mutations in the *APC* gene. In addition, mutation of *APC* is associated with more than 80% of sporadic colorectal adenomas and carcinomas. More than 95% of germline and somatic mutations of the *APC* gene are nonsense mutations that result in the synthesis of a truncated protein lacking the region of APC that is important for its function in the destruction complex. Significantly, these truncations in APC remove binding sites for β-catenin and Axin, as well as putative phosphorylation sites for GSK3; as a result, the mutant APC protein cannot efficiently promote degradation of β-catenin. Mutations in the third exon of the human β-catenin gene (*CTNNb1*) that make it refractory to phosphorylation-dependent degradation and lead to inappropriate accumulation of β-catenin have also been identified in a large number of primary human cancers (see [64] for a table of β-catenin mutations in human cancers). Interestingly, mutations in *CTNNb1* and *APC* are rarely found in the same tumor; for example, in colon cancer, in which the vast majority of tumors have mutations in *APC*, the overall frequency of *CTNNb1* mutations is relatively low, but colorectal tumors lacking *APC* mutations are much more likely to have mutations in *CTNNb1*. Recently, Axin has also been shown to act as a tumor suppressor; mutations in the *Axin1* gene have been found in human hepatocellular cancers [86]. Importantly, mutations in *Axin1* and *CTNNb1* found in hepatocellular carcinomas also show mutual exclusivity similar to that seen for *APC* and *CTNNb1* in colon cancers. Together, these data strongly argue that mutations resulting in the stabilization of β-catenin can promote cancer in many tissue types.

Frontiers

The large number of *Wnt* genes and the many roles that Wnt signaling plays in development and human disease pose many unresolved issues for researchers. One of the major unanswered questions is the specificity of interactions between different Wnt ligands and Fzd receptors and also which downstream pathways these many different ligand-receptor pairs stimulate. It also remains unclear how Wnt signals are transduced by the Fzd-LRP receptor complex and what role proteoglycans play in this process. Inside the cell, many questions regarding the transduction of Wnt signals remain, including how receptor activation stimulates Dsh and how Dsh discriminates between different Wnt signals to activate either the Wnt/β-catenin or the Wnt/polarity pathway. Furthermore, many roles of *Wnts* during development remain to be determined. This challenge will require detailed analyses of knockout mice, in addition to biochemical, cell-biological and genetic analyses in other model systems, to characterize the functions of Wnts and the signaling pathways they use during embryogenesis. Finally, the identification and characterization of mutations in Wnt-pathway genes involved in human disease is ongoing and these studies, together with a greater knowledge of the molecular mechanism of Wnt signal transduction, promise future clinical therapies for devastating human afflictions such as colon cancer. Thus, although there is so much still to learn, the importance and widespread occurrence of Wnt signaling guarantees the rapid increase in our understanding of the normal and abnormal functions of the Wnts.

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The authors describe experiments demonstrating that overexpression of Wnt5a in combination with human FZD5 promotes signaling through the Wnt/β-catenin pathway, suggesting that the specificity of cellular responses to different Wnt signals is regulated by the repertoire of Fzd receptors present on the cell surface of responding cells.
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The authors show that *swaying* phenotype is caused by deletion of a single base pair from Wnt1 that results in premature termination of translation, eliminating the carboxy-terminal half of the Wnt1 protein.
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A comprehensive review summarizing the role of Wnt signaling in the establishment of the dorsal-ventral axis in *Xenopus*.
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The authors show that reducing Fzd7 function with antisense oligonucleotides in early *Xenopus* embryos results in defects in dorsal development. These data suggest that Fzd7 plays a critical role in the specification of dorsal cell fates and provides circumstantial evidence that a Wnt ligand is also required for this process.
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This review summarizes our knowledge of the role of Wnt signaling in regulating early cell fate decisions in the soil worm, *C. elegans*. The review discusses the interesting findings that in *C. elegans* Wnt signals regulate cell fate choices via two pathways: one that involves activation of a mitogen activated protein kinase and another that involves a heterotrimeric G-protein and polarization of the mitotic spindle.
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This paper presents the sequence of the human *int-1* (*WNT1*) gene and compares the sequence and organization of the human and mouse *int-1* genes.
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The authors describe the cloning, sequence, and expression pattern of the mouse *Wnt2* gene. Adult expression of mouse *Wnt2* is restricted to lungs and heart, and fetal expression, to the pericardium of the heart, to the umbilicus and associated allantoic mesoderm, and to the ventral lateral mesenchyme tissue surrounding the umbilical vein.

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This paper describes the cloning, sequence and mapping of the human *WNT13* gene to 1p13. The authors also show that *WNT13* is expressed in several cell lines including HeLa (cervical cancer), MKN28 and MKN74 (gastric cancer) cells.

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This paper describes the cloning and expression of the mouse *Wnt13* gene. *Wnt13* is expressed in the embryonic mesoderm during gastrulation and, at later stages, transcripts are detected in the dorsal midline of the diencephalon and mesencephalon, the heart primordia, the periphery of the lung bud and the otic and optic vesicles.

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The authors describe the characterization of the mouse *Wnt3* gene on chromosome 11 as a site for mouse mammary tumor pro-virus insertion. The paper also presents expression data for *Wnt3.*

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This paper describes the genomic localization of the human *WNT3* gene close to a CA repeat marker on chromosome 17q.

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This paper describes the characterization of the human *WNT3* gene and its localization to chromosome 17q21. Analysis of the *WNT3* gene in a collection of mammary tumor samples failed to detect rearrangements or amplification.

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The authors report the expression of *WNT3*, *WNT4*, and *WNT7b* in human breast cell lines and *WNT2*, *WNT3*, *WNT4*, and *WNT7b* in human breast tissues. *WNT3a* and *WNT7a* were not expressed in of the examined tissue. In addition, several of these genes, including *WNT2*, *WNT4* and *WNT7b*, showed increase expression in breast tumors.

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The authors present genetic and expression analyses demonstrating that the *vestigial tail* mutation is a hypomorphic allele of *Wnt3a.*

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This paper describes the sequence, expression, and mapping of the human *WNT3A* and *WNT14* genes. The genes were localized to chromosome 1q42 in a head to head manner separated by approximately 58 kb.

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105. Clark CC, Cohen I, Eichstetter I, Cannizzaro LA, McPherson JD, Wasmuth JJ, Iozzo RV: **Molecular cloning of the human proto-oncogene Wnt-5A and mapping of the gene (WNT5A) to chromosome 3p14-p21**. *Genomics* 1993, **18:**249-260.
This paper describes the cloning and mapping of the human *WNT5A* gene. RT-PCR expression analysis of a variety of embryonic, neonatal, and adult cells and/or tissues showed that *WNT5A* was detected only in neonatal heart and lung.

106. Adamson MC, Dennis C, Delaney S, Christiansen J, Monkley S, Kozak CA, Wainwright B: **Isolation and genetic mapping of two novel members of the murine Wnt gene family, Wnt11 and Wnt12, and the mapping of Wnt5a and Wnt7a.** *Genomics* 1994, **24:**9-13.
The authors cloned the mouse *Wnt11* and *Wnt12* (*Wnt10b*) genes by degenerate PCR and mapped both of these *Wnt* genes as well as the *Wnt5a* and *Wnt7a* genes. *Wnt11* mapped to chromosome 7, *Wnt12* (*Wnt10b*) to chromosome 15 close to *Wnt1*, *Wnt5a* to chromosome 14, and *Wnt7a* to chromosome 6.

107. Saitoh T, Katoh M: **Molecular cloning and characterization of human WNT5B on chromosome 12p13.3 region.** *Int J Oncol* 2001, **19:**347-351.
Expression analysis shows that *WNT5A* is expressed in adult prostate and fetal brain, and weakly expressed in fetal lung, kidney, adult liver, ovary, and small intestine. *WNT5B* is also expressed in the gastric cancer cell lines MKN7, MKN45, KATO-III, and a teratocarcinoma cell line NT2.

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This paper reports the cloning of a partial cDNA encoding *WNT6* and its mapping to chromosome 2q35.

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This paper describes the cloning and mapping of human *WNT7A.*

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113. Kirikoshi H, Sekihara H, Katoh M: **Molecular cloning and characterization of human WNT7B.** *Int J Oncol* 2001, **19:**779-783.
The authors describe the characterization of the human *WNT7B* gene and its expression in fetal brain, lung and kidney, and in adult brain, lung and prostate. *WNT7B* is also expressed in a lung cancer cell line A549, esophageal cancer cell lines TE2, TE3, TE4, TE5, TE6, TE7, TE10, TE12, a gastric cancer cell line TMK1, and pancreatic cancer cell lines BxPC-3, AsPC-1 and Hs766T. In addition, *WNT7B* was found to be up regulated in 50% of primary gastric cancers.

114. Bouillet P, Oulad-Abdelghani M, Ward SJ, Bronner S, Chambon P, Dolle P: **A new mouse member of the Wnt gene family, mWnt-8, is expressed during early embryogenesis and is ectopically induced by retinoic acid.** *Mech Dev* 1996, **58:**141-152.
The authors describe the cloning of the mouse *Wnt8* gene and its expression during embryogenesis. *Wnt8* is expressed in the posterior region of the epiblast of early primitive streak-stage embryos and as gastrulation proceeds expression spreads into the embryonic ectoderm. *Wnt8* is also transiently expressed in the mesoderm.

115. Saitoh T, Katoh M: **Molecular cloning and characterization of human WNT8A.** *Int J Oncol* 2001, **19:**123-127.
This paper presents the sequence and organization of the human *WNT8A* gene. Expression analysis of *WNT8A* in various human tissues and cell lines only detected transcripts in NT2 teratocarcinoma cells.

116. Lako M, Strachan T, Curtis AR, Lindsay S: **Isolation and characterization of WNT8B, a novel human Wnt gene that maps to 10q24**. *Genomics* 1996, **35:**386-388.
This paper and [117] describe the cloning, mapping, and expression analysis of the human *WNT8B* gene and [117] presents expression data for the mouse *Wnt8b* gene. Both the human and mouse *Wnt8b* genes were restricted to the developing brain, with the majority of expression being found in the forebrain.

117. Lako M, Lindsay S, Bullen P, Wilson DI, Robson SC, Strachan T: **A novel mammalian wnt gene, WNT8B, shows brain-restricted expression in early development, with sharply delimited expression boundaries in the developing forebrain.** *Hum Mol Genet* 1998, **7:**813-822.
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The authors present the mapping of mouse *Wnt8b* and characterize its expression in the developing forebrain. See also [117].

119. Wang J, Shackleford GM: **Murine Wnt10a and Wnt10b: cloning and expression in developing limbs, face and skin of embryos and in adults.** *Oncogene* 1996, **13:**1537-1544.
The authors report the isolation of the mouse *Wnt10a* and *Wnt10b* genes as well as analyses of the expression patterns of these genes in adult and embryonic tissues. In adults, *Wnt10a* RNA was most abundant in adult brain with a high concentration in the pituitary gland, *Wnt10b* was highest in lung and uterus, and mRNAs of both genes were detected in thymus and spleen. In embryos, expression was found in a variety of tissues including limbs, face, skin, and liver.

120. Christiansen JH, Dennis CL, Wicking CA, Monkley SJ, Wilkinson DG, Wainwright BJ: **Murine Wnt-11 and Wnt-12 have temporally and spatially restricted expression patterns during embryonic development.** *Mech Dev* 1995, **51:**341-350.
This paper describes the expression patterns of mouse *Wnt11* and *Wnt12* (*Wnt10b*). *Wnt11* expression is first detected within the truncus arteriosus and is later detected in the somites at the junction of the dermatome and the myotome and in limb bud mesenchyme. *Wnt12* (*Wnt10b*) is also expressed in the limb and is expressed in the apical ectodermal ridge.

121. Lee FS, Lane TF, Kuo A, Shackleford GM, Leder P: **Insertional mutagenesis identifies a member of the Wnt gene family as a candidate oncogene in the mammary epithelium of int-2/Fgf-3 transgenic mice.** *Proc Natl Acad Sci USA* 1995, **92:**2268-2272.
This paper presents the characterization of the mouse *Wnt10b* gene as a site of mouse mammary tumor virus insertion in int-2/Fgf-3 transgenic mice that cooperate with int-2/Fgf-3 in tumorigenesis. The authors also showed that *Wnt10b* is expressed in the embryo and mammary gland of virgin but not pregnant mice. See also [123].

122. Bui TD, Rankin J, Smith K, Huguet EL, Ruben S, Strachan T, Harris AL, Lindsay S: **A novel human Wnt gene, WNT10B, maps to 12q13 and is expressed in human breast carcinomas.** *Oncogene* 1997, **14:**1249-1253.
This paper describes the characterization of the human *WNT10B* gene showing that it maps to 12q13 in close proximity to *WNT1*. The authors also examine the expression of *WNT10b* in human breast cancers. See also [124].

123. Hardiman G, Albright S, Tsunoda J, McClanahan T, Lee F: **The mouse Wnt-10B gene isolated from helper T cells is widely expressed and a possible oncogene in BR6 mouse mammary tumorigenesis.** *Gene* 1996, **172:**199-205.
This paper describes the cloning of mouse *Wnt10b* and its expression in embryos and adults. In addition, the *Wnt10b* gene is shown to be an insertion site for mouse mammary tumor virus an may contribute to mammary tumors in BR6 mice. See also [121].

124. Hardiman G, Kastelein RA, Bazan JF: **Isolation, characterization and chromosomal localization of human WNT10B.** *Cytogenet Cell Genet* 1997, **77:**278-282.
This paper presents the cloning and mapping of the human *WNT10B* gene to 12q13. The expression pattern of *WNT10B* reveals that it is present in many adult tissues, with the highest levels found in heart and skeletal muscle, and is also expressed in several human cancer cell lines, including HeLa cells. See also [122].

125. Lako M, Strachan T, Bullen P, Wilson DI, Robson SC, Lindsay S: **Isolation, characterisation and embryonic expression of WNT11, a gene which maps to 11q13.5 and has possible roles in the development of skeleton, kidney and lung.** *Gene* 1998, **219:**101-110.
The authors characterize the human *WNT11* gene, mapping it to 11q13.5 and demonstrating its expression in the perichondrium of the developing skeleton, lung mesenchyme, the tips of the ureteric buds and other areas of the urogenital system and the cortex of the adrenal gland.

126. Bergstein I, Eisenberg LM, Bhalerao J, Jenkins NA, Copeland NG, Osborne MP, Bowcock AM, Brown AM: **Isolation of two novel WNT genes, WNT14 and WNT15, one of which (WNT15) is closely linked to WNT3 on human chromosome 17q21**. *Genomics* 1997, **46:**450-458.
This paper describes the cloning and mapping of human *WNT14* and *WNT15* and show that *WNT13* (*WNT2B*) is expressed in mammary tissue.

127. McWhirter JR, Neuteboom ST, Wancewicz EV, Monia BP, Downing JR, Murre C: **Oncogenic homeodomain transcription factor E2A-Pbx1 activates a novel WNT gene in pre-B acute lymphoblastoid leukemia.** *Proc Natl Acad Sci USA* 1999, **96:**11464-11469.
The authors characterize the human *WNT16* gene and show that it is activated by the E2A-Pbx1 fusion protein in pre-B acute lymphoblastoid leukemia. *WNT16* is normally expressed in the spleen, appendix, and lymph nodes, but not in bone marrow. However, *WNT16* transcripts are highly expressed in bone marrow and cell lines derived from pre-B ALL patients carrying the E2A-Pbx1 fusion suggesting that inappropriate expression of *WNT16* plays a role in leukemia.

128. Fear MW, Kelsell DP, Spurr NK, Barnes MR: **Wnt-16a, a novel Wnt-16 isoform, which shows differential expression in adult human tissues.** *Biochem Biophys Res Commun* 2000, **278:**814-820.
The authors map human *WNT16* to 7q31 and characterize the differential expression of two distinct *WNT16* isoforms. The isoforms were shown to utilize different 5'-UTRs and first exons.

129. McMahon AP, Gavin BJ, Parr B, Bradley A, McMahon JA: **The Wnt family of cell signalling molecules in postimplantation development of the mouse.** *Ciba Found Symp* 1992, **165:**199-212.
This paper summarizes the phenotype of the *Wnt1* knockout mice. See [74].

130. Mastick GS, Fan CM, Tessier-Lavigne M, Serbedzija GN, McMahon AP, Easter SS, Jr.: **Early deletion of neuromeres in Wnt-1$^{-/-}$ mutant mice: evaluation by morphological and molecular markers.** *J Comp Neurol* 1996, **374:**246-258.
This paper builds on [74] and provides a detailed characterization of the phenotype of *Wnt1* deficient mice focusing on possible perturbations in structures adjacent to the presumptive midbrain and cerebellum.

131. Ikeya M, Lee SM, Johnson JE, McMahon AP, Takada S: **Wnt signalling required for expansion of neural crest and CNS progenitors.** *Nature* 1997, **389:**966-970.
The authors show that mice mutant for both *Wnt1* and *Wnt3a* show a dramatic decrease in the number of neural crest progenitors, normally derived from the dorsal neural tube.

132. Monkley SJ, Delaney SJ, Pennisi DJ, Christiansen JH, Wainwright BJ: **Targeted disruption of the Wnt2 gene results in placentation defects.** *Development* 1996, **122:**3343-3353.
This paper examines the phenotype of *Wnt2* knockout mice and show that mice lacking *Wnt2* display runting and approximately 50% died perinatally. Mutant mice were found to have defects in the size and structure the placenta with notable perturbation of the vascularization of the placenta.

133. Millar SE, Willert K, Salinas PC, Roelink H, Nusse R, Sussman DJ, Barsh GS: **WNT signaling in the control of hair growth and structure.** *Dev Biol* 1999, **207:**133-149.
This paper shows that overexpression of *Wnt3* in skin of transgenic mice results in a short hair phenotype implicating Wnt signaling in hair growth. Overexpression of Dishevelled-2 (Dvl2) in outer root sheath cells mimicked this phenotype.

134. Kishimoto J, Burgeson RE, Morgan BA: **Wnt signaling maintains the hair-inducing activity of the dermal papilla.** *Genes Dev* 2000, **14:**1181-1185.
The authors show that specific *Wnt* genes can maintain anagen-phase gene expression in isolated dermal papilla cells in vitro and hair inductive activity in a skin reconstitution assay.

135. Takada S, Stark KL, Shea MJ, Vassileva G, McMahon JA, McMahon AP: **Wnt-3a regulates somite and tailbud formation in the mouse embryo.** *Genes Dev* 1994, **8:**174-189.
This paper and [136] describe the phenotype of mice lacking the *Wnt3a* gene. *Wnt3a*$^{-/-}$ embryos lack caudal somites, have a disrupted notochord, and fail to form a tailbud. Mutant mice also possess an ectopic neural tube suggesting that *Wnt3a* plays a critical role in specifying paraxial mesoderm and that in its absence these cells adopt neural fates.

136. Yoshikawa Y, Fujimori T, McMahon AP, Takada S: **Evidence that absence of Wnt-3a signaling promotes neuralization instead of paraxial mesoderm development in the mouse.** *Dev Biol* 1997, **183:**234-242.
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