REVIEW ARTICLE
Signal transduction by the Wnt family of ligands

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The Wnt genes encode a large family of secreted polypeptides that mediate cell–cell communication in diverse developmental processes. The loss or inappropriate activation of Wnt expression has been shown to alter cell fate, morphogenesis and mitogenesis. Recent progress has identified Wnt receptors and components of an intracellular signalling pathway that mediate Wnt-dependent transcription. This review will highlight this 'core' Wnt signal-transduction pathway, but also aims to reveal the potential diversity of Wnt signalling targets. Particular attention will be paid to the overlap between developmental biology and oncogenesis, since recent progress shows Wnt signalling forms a paradigm for an interdisciplinary approach.

INTRODUCTION

Wnt proteins are a large family of cysteine-rich secreted ligands that control development in organisms ranging from nematode worms to mammals. Recent data suggests that deregulated Wnt signalling may also be involved in tumour formation. At the cellular level, Wnts regulate cell proliferation, cell morphology, cell motility and cell fate. A major goal of recent studies has been to understand how Wnt regulation of cellular processes translates to the developmental/morphological level. Experimental approaches have focused on intracellular signalling, with the aim of generating mechanistic links between Wnt binding to its receptors and cellular outcomes. Several components of a signalling pathway linking ligand binding at the cell surface to transcription in the nucleus have now been identified [1,2]. The order of topics within this review follows the cell surface-to-nucleus order of known signalling components established for the canonical pathway (Scheme 1). An important theme of Wnt signalling is the conservation of canonical signalling mechanisms through evolution. In order to highlight this point, generic names of Wnt signalling components (as used in Scheme 1) will be used. However, detailed analyses point to important differences between experimental systems, a point that may in part be due to the plethora of gene families corresponding to each signalling component (see Tables 1a and 1b). It is important to emphasize from the outset that not all parts of the molecular chain leading to Wnt-induced transcription have been identified, and that Wnt-induced signals probably diverge from the consensus pathway to target different cellular processes such as cell adhesion and cell motility.

Wnt TO FRIZZLED

Wnts are a family of cysteine-rich glycosylated ligands (more than 16 mammalian family members) that bind to the extracellular domain of Frizzled receptors (more than eight mammalian family members; Table 1b). The Frizzled genes are named after the *Drosophila* tissue polarity gene *frizzled* (*fz*), which was the first member to be isolated (Table 1b). Structurally, Frizzled receptors have an extracellular Wnt-binding domain, seven-transmembrane-spanning sequences and an intracellular C-terminal tail (Figure 1 below). They form part of the seven-transmembrane-spanning (7TMS) superfamily of receptors. The regulation of Wnt binding to Frizzled receptors is a key restriction point in Wnt signalling.

Wnts bind Frizzled receptors and activate signalling

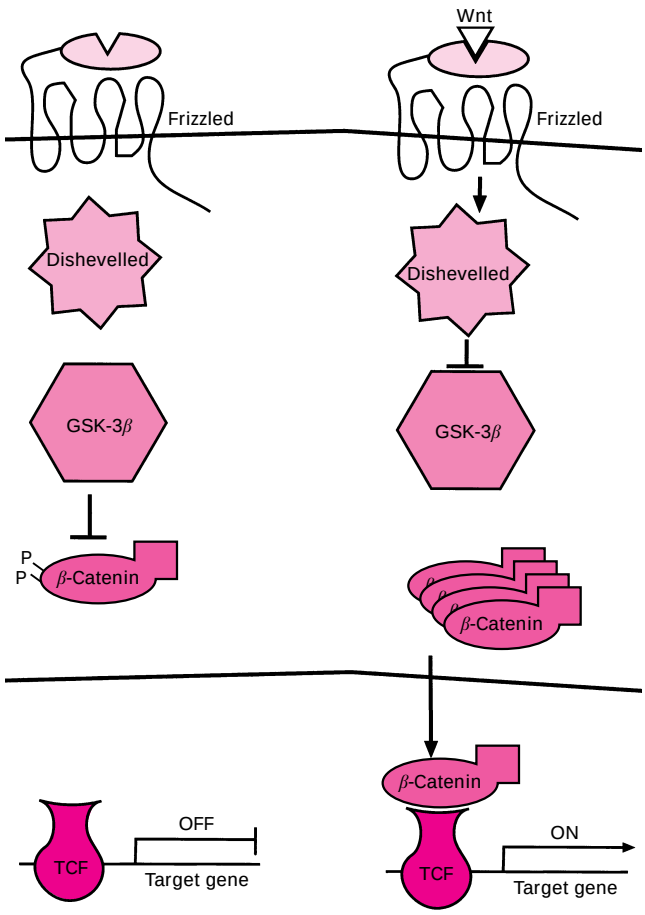
The first demonstration that 7TMS Frizzled proteins functioned as Wnt receptors came from studies in the *Drosophila* S2 cell line. Following expression of *Drosophila frizzled* 2 (*Dfz2*), S2 cells attained the ability to bind Wg protein (Wingless; DWnt-1) and activated Wg signalling, as shown by the stabilization of intracellular levels of the Armadillo (Arm; see Table 1b; [3]) protein. Parental S2 cells lacked *Dfz2* expression and were unable to respond to soluble Wg. Additional analyses suggested a large extracellular N-terminal domain of the Frizzled receptor was necessary and sufficient for Wg binding [3]. Biochemical evidence for direct Wnt–Frizzled interactions came from *in vitro* studies showing Xwnt-8 bound to Frzb-1, a soluble protein containing a single 'Wnt binding domain' with similarity to the extracellular region of Frizzled family members (see below; reviewed in [1,4,5]).

Additional functional assays linking Wnt–Frizzled binding to downstream signalling came from studies of the Wnt-induced phenomenon of axis duplication in *Xenopus* embryos in which the induction of the Siamois DNA-binding protein is the key transcriptional target (Table 1a). In these studies Xwnt-8-activated expression of Siamois through RFz1 (rat Frizzled 1)- and Xwnt-5A-induced axis duplication through HFz5 (human Frizzled 5) [6,7].

Receptor–ligand specificity

The binding specificity of Wg–Frizzled interactions was determined by expressing a range of mammalian Frizzled cDNAs in 293 cells and measuring the cell-surface retention of soluble Wg. These assays showed that fz (*Drosophila* Frizzled 1), Dfz2, Mfz4 (mouse Frizzled 4), Hfz5, Mfz7 and Mfz8, but not Mfz3, bound Wg [3]. As 14 Wnt and eight Frizzled mammalian genes

Abbreviations used: Wnt, member of the Wnt family of peptide ligands; Fz, Frizzled receptor; Frzb, secreted protein family containing Wnt-binding domains; Dsh, Dishevelled family member; GSK-3, glycogen synthase kinase-β; Arm, Armadillo member of the β-catenin family; TCF, member of the T-cell factor family of HMG (high-mobility group)-box DNA-binding proteins; 7TMS, seven-transmembrane-spanning receptors; FGF, fibroblast growth factor; TGF-β, transforming growth factor-β; JNK, Jun kinase; SAPK, stress-activated protein kinase; SRF, serum response factor; PKC, protein kinase C; dn, as in dnGSK-3, dominant negative; (D-)APC, (*Drosophila*) adenomatous polyposis coli; EGF, epidermal growth factor; dpp, decapentaplegic.



Scheme 1 The canonical Wnt signalling pathway

Following binding of the Wnt ligand to the Frz receptor, Dsh is recruited to the cell membrane, where it signals to inhibit the kinase activity of GSK-3. As GSK-3 normally promotes the instability of soluble β-catenin, Wnt signalling results in an increase of β-catenin levels. Soluble β-catenin then interacts with TCF-DNA-binding factors, forming a transcriptionally active complex. In several versions of this model, the *APC* (adenomatous polyposis coli) gene product has been proposed to function as a signalling intermediate between GSK-3 and β-catenin.

have been identified (Table 1b), much remains to be learnt about the spectrum of Wnt–Frizzled binding interactions.

Prior to the discovery of Frizzled receptors, results from ectopic expression of Wnts in mammary epithelial cells and *Xenopus* embryos were used to divide Wnts into a transforming, axis-inducing 'Wnt-1 class' (murine Wnt-1, -3A and -7A; *Xenopus* Xwnt-1, -3A, -8 and 8b) and a non-transforming 'Wnt-5A' class (murine Wnt-4, and -5A; *Xenopus* Xwnt-5A and Xwnt-4) [8–11]. The use of Xwnt-8:Xwnt-5A chimaeric Wnts showed that Wnt subtype-specificity determinants were localized in the C-terminus of the molecule [11] (Figure 1).

Whereas Wnt-Frizzled binding specificity may underly many 'Wnt-class' specific effects, it does not precisely reflect the simple division of Wnts into two classes, since Hfz5 bound Wg (Wnt-1 class) and also functioned as a receptor for Xwnt-5A (Wnt-5A class) [3,7]. These data raise the possibility that receptor binding may not be sufficient for activation and question the assumption that Wnts can be divided into just two classes.

Redundancy and competition. As Wg bound six out of the seven Frizzled receptors tested [3], it is probable that individual Frizzled receptors are capable of binding multiple Wnts and that many Wnts will bind multiple receptors. Studies of fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β)/activin ligands showed similar redundancy in receptor binding [12–14]. Endogenous Wnt expression patterns in systems such as the mouse hindbrain overlap, raising the possibility that Wnts may functionally substitute for each other *in vivo* [15,16]. Redundancy of receptor binding may underly the common observation that developmental defects in Wnt null mice occur in only a subset of the regions in which the Wnt is expressed [17–20]. Similarly, redundancy by Wg in the activation of *Dfz2* and *frizzled* (*fz*) may explain why Frizzled genes were not isolated during extensive screens for segment polarity class mutants in *Drosophila* epidermal development [3].

In addition to co-activation, redundant sets of Wnts may interfere with each other's function. Evidence from studies of Spitz and Argos binding to the *Drosophila* epidermal-growth-factor (EGF) receptor and Delta and Serrate binding to the Notch receptor have shown that ligands may function by mutually antagonizing each other's activity [21,22]. A C-terminal deletion of Xwnt-8 (see Figure 1) generated a dominant negative mutant (dnXwnt-8) that interfered with endogenous Xwnt-8 function, showing Wnt binding can be decoupled from Wnt signalling [23].

Evidence for Wnt–Wnt interference has most clearly been demonstrated in studies of axis duplication in *Xenopus* embryos, where prior expression of Xwnt-5A blocked axis duplication by Xwnt-8 [8]. However, it is not clear whether XWnt-5A blocked Xwnt-8 action by receptor competition, by changing cell adhesion or by blocking the action of intracellular signalling components [24]. Interestingly, XWnt-5A induced axis duplication comparable with that of Xwnt-8 when co-expressed with Hfz5, [7]. Although it is clear that Hfz5 can couple to the canonical Wnt pathway, it is unclear how Xwnt-5A regulates morphogenetic movements when expressed alone. One possibility is that endogenous Xfrizzled receptors couple Xwnt-5A to morphogenetic effector pathways (i.e. non-canonical). Alternatively, the endogenous Xwnt-5A receptor may couple to the canonical pathway, but may not be expressed at the appropriate time or place to mediate axis duplication.

Ligand availability

The regulation of Wnt availability or presentation to Frizzled receptors offers an additional route for regulation during Wnt signalling. The response to Wnt availability may be more than a simple on/off switch, since studies in *Drosophila* showed that Wg up-regulated the transcription of particular genes as a function of concentration. Discrete responses to different levels of Wnt protein may also be reflected in the observation that graded amounts of the downstream Dishevelled protein specified distinct cellular fates during *Xenopus* embryogenesis (see below and [25]).

Frzb

The 'Wnt-binding domain' found at the N-terminus of Frizzled family members was recently identified in genes of the Frzb family and in a splicing variant of collagen XVIII (reviewed in [4,26,27,27a,28]). Frzb proteins lacked the 7TMS sequences found in their signalling counterparts and were secreted. Frzb-1 was shown to bind Xwnt-8 and inhibit its ability to induce an ectopic axis [4,28]. The effect of ectopic Frzb-1 expression on endogenous patterning was very similar to that observed for dnXwnt-8, suggesting that Frzb-1 functioned by sequestering Xwnt-8 in an inactive complex. As both Frzb-1 and Wnts are

Table 1 (a) Systems used to analyse Wnt signalling and (b) Wnt signalling components in different systems

While Wnt function has been analysed in a large number of systems, only a subset of these have been used to analyse Wnt signalling (a). The first evidence for a Wnt signalling pathway came from studies of *Drosophila* ventral epidermal development [226,227] (b). The most persuasive support for the canonical signalling pathway came from studies of axis formation in *Xenopus*, where ectopic Wnt signals generated two-headed embryos (reviewed in [228]) (b). In this system, Wnts transcriptionally regulated the induction of the Siamois DNA-binding protein[194,229,230], However, molecular components upstream of β-catenin may not be required for the establishment of the endogenous *Xenopus* axis [118,150,151,195]. Elements upstream of β-catenin may be involved in the later induction of dorso-anterior structures and the induction of MyoD [23]. The recent demonstration of soluble Wg activity has allowed the biochemical analysis of early stages of Wnt signalling in cell-culture models[78,231]. These studies are complemented by studies of tumour-cell lines in which Wnt signalling may have been activated by mutation. Note that reviews documenting gene families are given in preference to original references.

(a) Systems used to analyse Wnt signalling

Organism	Stage/system	Ectopic Wnt + phenotype	Molecular targets
Xenopus	Early blastula (Nieuwkoop Centre)	Axis duplication leading to two-headed tadpoles	Siamois DNA-binding protein [208]
Xenopus	Mid-gastrula	Suppression of dorsoanterior structures; induction of somite formation	MyoD [23]
Drosophila	Ventral epidermis	Naked cuticle	Engrailed DNA-binding protein
Drosophila	Wing margin bristles	Increased bristles	Notch binding by Dishevelled? [47]
Drosophila	Eye morphology	Rough eyes	[209]
Drosophila	Cl-8 cell line	Not applicable	Armadillo stabilization [114]
C. elegans	Gut induction	Loss of Wnt signal generates gutless embryos	POP-1 [59]
C. elegans	T- and B-cell polarity?	Altered polarity	POP-1? [190]
Mouse	Cell lines	'Morphological transformation'; cellular proliferation	β-Catenin stabilization GSK-3 inhibition [65,116,147]
Human	Tumour-cell lines	Tumour lines are already assumed to be positive for Wnt signalling, thus assays inhibit Wnt signalling components and measure the suppression of proliferation?	TCF-dependent transcription [122,176]

(b) Wnt signalling components in different systems

Generic	Mammalian	*Drosophila*	*C. elegans*	*Xenopus*
Wnt	Wnt $\geqslant$ 14 members [210,211]	Dwnt (Dwnt-1 = wg) $\geqslant$ five members [212–215]	Lin-44, MOM-2 [57,60,61]	Xwnt $\geqslant$ 13 members [216,217]
Frizzled (Fz)	Fz $\geqslant$ eight members [218,219]	fz $\geqslant$ two members [3,202]	Lin-17, MOM-5 [58,60]	Fz none yet described
Dishevelled (Dsh)	Dvl $\geqslant$ three members [70,220,221]	dsh 1 gene [51,68]	(possible two members [60])	Xdsh $\geqslant$ 1 gene [84]
GSK-3	GSK-3β α- and β-isoforms [82]	zw-3/sgg one gene [82]		XGSK-3 β-isoform [91,92]
APC	APC $\geqslant$ one gene [222]	dAPC $\geqslant$ 1 gene [182]	APR-1 [60]	XAPC $\geqslant$ 1 gene [183]
β-Catenin	β-Catenin $\geqslant$ two genes [138,139,223]	arm 1 gene [224]	WRM-1 [60]	β-catenin $\geqslant$ two genes [225]
TCF	TCF/LEF $\geqslant$ four genes [187–189]	dTCF/pangolin $\geqslant$ one gene [135,191]	POP-1 [190]	XTCF $\geqslant$ three isoforms XTCF3 [185]

secreted factors, gradients of both may act to pattern structures by generating regional thresholds of free Wnt ligand [4,28,29].

Co-receptors

The complexity of receptor–ligand specificity may be further increased by co-receptors such as proteoglycans and other putative 'Wnt receptors'.

Proteoglycans. Proteoglycans consist of proteins linked to chains of disaccharide repeats called glycosaminoglycans. Proteoglycans are highly charged and are found predominantly at the cell surface, where they function as low-affinity cell-surface receptors for a variety of ligands, including TGF-β and FGF [30,31]. Cell-culture studies showed that many Wnt family members bind glycosaminoglycans [32–34] and that cell-surface glycosaminoglycans are required for Wg stabilization of Arm in *Drosophila* Cl-8 cells (Table 1a) [35]. The particular importance of glycosaminoglycans for Wnt signalling was emphasized by the finding that embryonic injection of the heparin-degrading enzyme heparinase produced a 'wingless-like' embryonic cuticular phenotype [36]. These studies were also supported by the finding that mutants in the *kiwi/ska* gene, which codes for the enzyme UDP-glucose dehydrogenase, had patterning defects similar to those of Wg mutants [36,37].

Cell-surface proteoglycans may increase the local concentration of Wnts, leading to increased avidity and/or receptor clustering, as has been proposed in models for FGF–glycosaminoglycan interactions [31]. Thus the loss of heparin-like glycosaminoglycans may allow Wnt proteins to abnormally diffuse and reach a concentration at which they are no longer effective. Alternatively, the ability of Wnt–Frizzled complexes to form or transduce their signals may be directly regulated by glycosaminoglycan interactions. During normal development, the control of glycosaminoglycan turnover (for example, see [38]) may offer indirect routes to alter Wnt signalling through changes in ligand availability or diffusion.

As well as binding glycosaminoglycans, Wnt proteins themselves are glycosylated [33,34,39–41]. Studies of Wnt-1 mutants lacking glycosylation motifs showed that glycosylation was essential for mammary epithelial transformation [42].

Notch. Complex genetic interactions argue that the single transmembrane receptor Notch genetically interacts with Wg [43–46]. However, as Notch bound intracellularly to Dishevelled

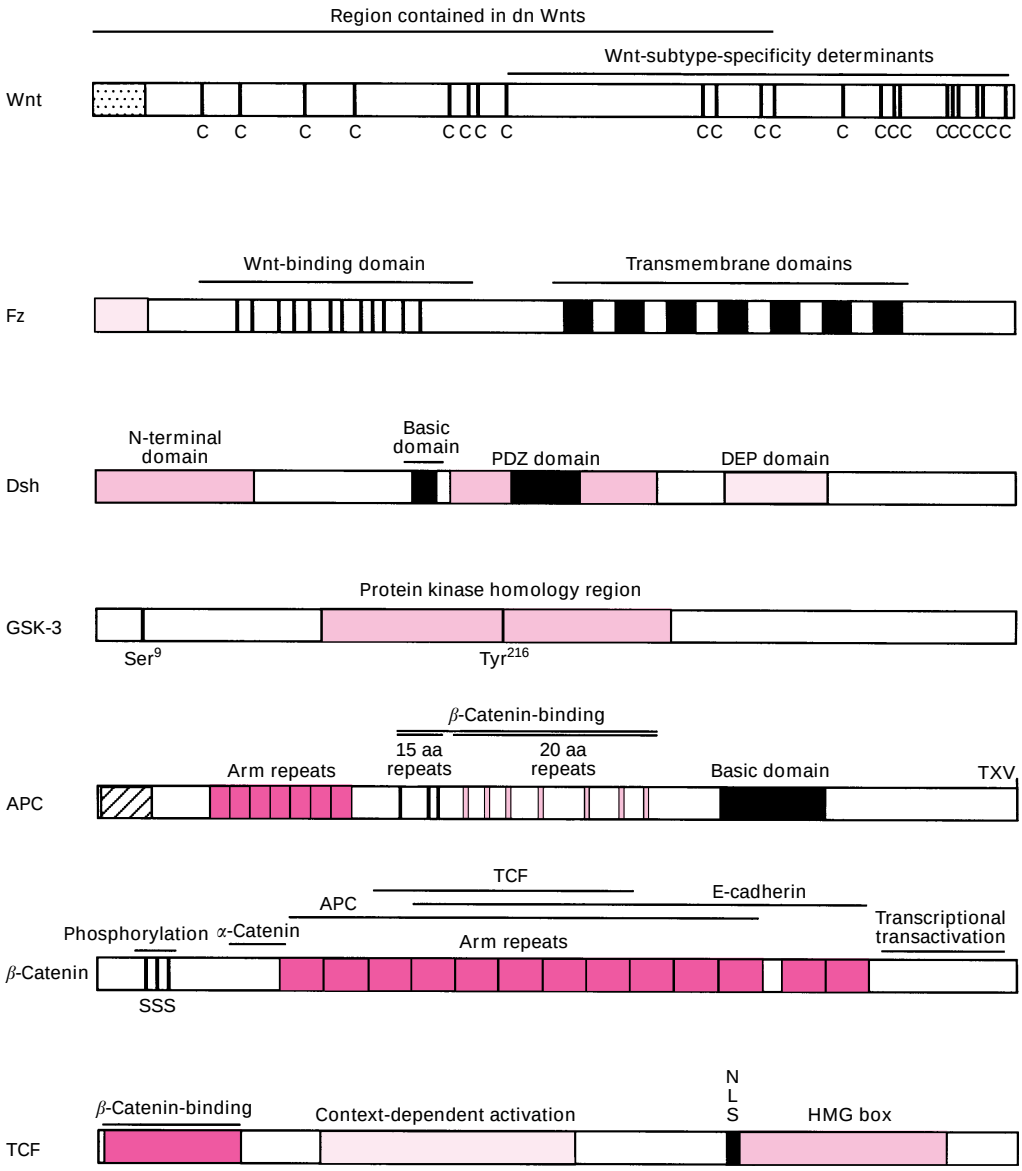


Figure 1 Domains and features of Wnt signalling components

Wnt: for dnWnt regions, see [23]; for subtype specificity, see [11]; murine Wnt-1, 370 amino acids; Fz, see [3,202]; Dfz2, 694 amino acids; Dsh, see [80]; Dsh, 623 amino acids; GSK-3 β, see [82]; rat GSK-3β, 440 amino acids; APC, see [175]; TXV, C-terminal sequence that may bind Dlg [203]; human APC, 2843 amino acids; β-catenin, for phosphorlation sites, see [119]; for α-catenin binding, see [130,134,204]; for binding over Arm repeats, see [132,135,205]; human β-catenin, 781 amino acids; TCF, see [135]; murine LEF-1, 394 amino acids. Abbreviations: aa, amino acids; NLS, nuclear localization signal.

(Dsh; see below) [47], it was difficult to distinguish whether Wg directly or indirectly interacted with Notch. Wg binding assays in 293 cells failed to detect Notch–Wg interactions [3]; however, these experiments were not performed in the presence of Ca^{2+}, which is required for Notch function. It thus remains possible that Notch binds Wg directly or that Notch acts as a Wg co-receptor for some or all Wg signalling functions.

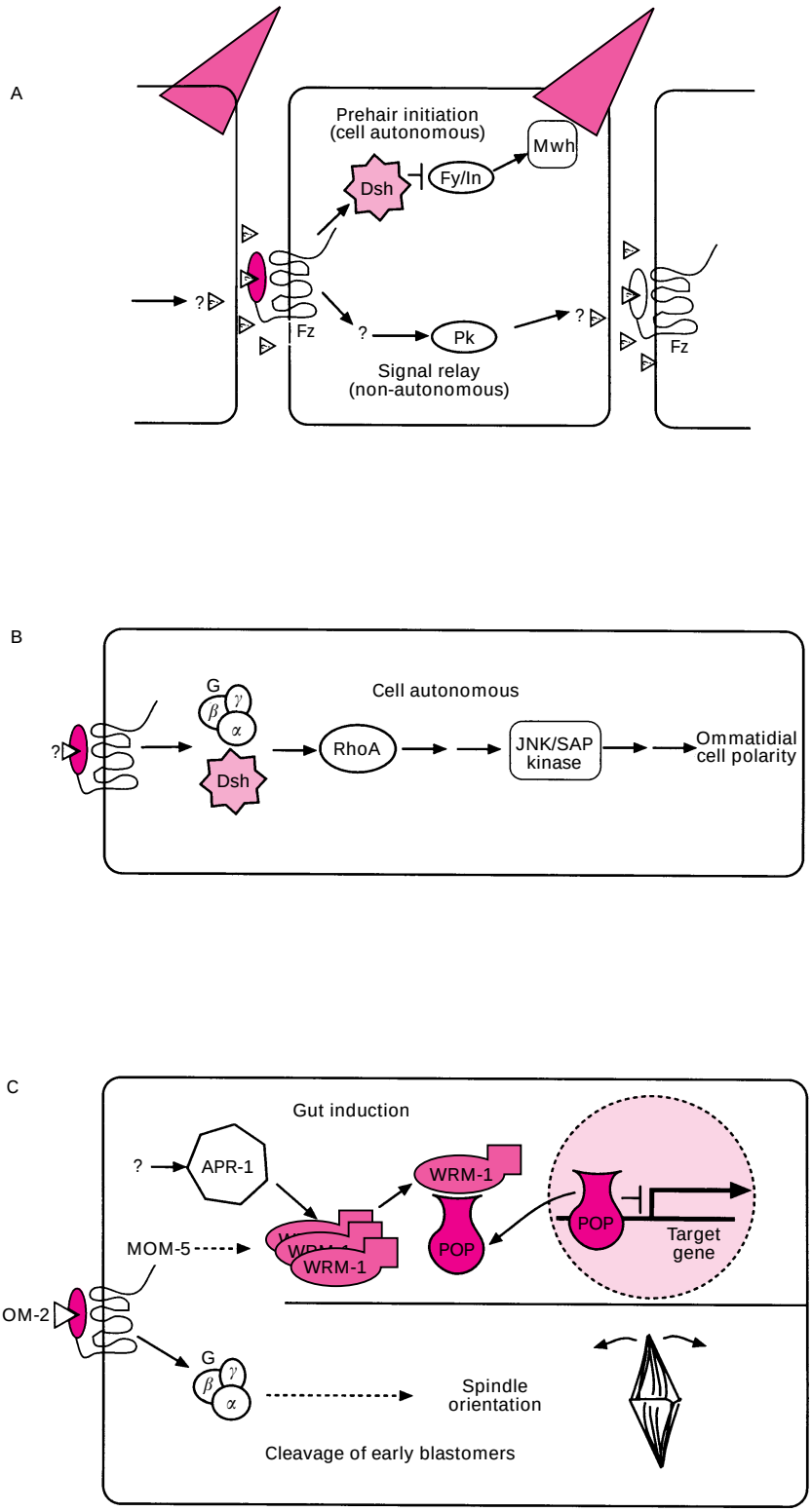
Non-canonical pathways

While it is clear that Frizzleds can function as Wnt receptors, it has not yet been demonstrated that Frizzleds always function as Wnt receptors. In the cases outlined below, Frizzled and/or Wnt family members are implicated in signalling pathways that are clearly different from the canonical Wnt pathway.

Drosophila tissue polarity

The tissue-polarity genes in *Drosophila* are required to co-ordinate the local orientation of cells with respect to their neighbours within the planar epithelium. Several genes that disrupt the polarity of bristles, hairs or ommatidia, but not the structure of the developmental unit itself, have been characterized and include *fz*, *prickle* (*pk*), *nemo* (*nmo*), *dsh*, *inturned* (*in*), *fuzzy* (*fy*), *multiple wing hair* (*mwh*), *RhoA* and *basket* (*bsk*) [48,49]. There is some disagreement over whether genetic evidence supports or excludes a role for Wg in tissue [48,50,51]; however, it is clear that Fz can bind Wg [3].

During the formation of wing-hair polarity, Fz has been proposed to regulate a branched intracellular signalling pathway in response to an unknown ligand (see Scheme 2A). Recent



Scheme 2 Signalling by members of the Frizzled superfamily

(**A** and **B**) The orientation of *Drosophila* wing hairs and ommatidia were disturbed by mutations in, or overexpression of, the tissue-polarity class of genes. Genetic epistatic analysis ordered components into the pathways shown. (**A**) Wing-hair polarity pathway. Fz, Frizzled; Fy, Fuzzy; In, Inturned; Msh, Multiple wing hair; Pk, Prickle (adapted from the Figure in [50] with permission of the The Company of Biologists). (**B**) Ommatidial polarity pathway. This pathway also operates in the wing (see above). However, the relationship between Rho A, the JNK homologue bsk and Fy/In was not investigated [48]. Prickle did not interact with Fz [48]. JNK, c-Jun kinase (*Drosophila* gene, bsk); Rho A, RhoA p21 GTPase. G$\alpha/\beta/\gamma$, heterotrimeric G protein proposed to be in pathway by analogy with the yeast-mating-pheromone pathway [48]. (**C**) Wnt signalling in early *C. elegans* development. MOM-2 (Wnt), MOM-5 (Fz), APR-1 (APC), WRM-1 (β-catenin) and POP-1 (TCF) function in a signalling pathway leading to gut induction [59–61]. In this pathway APR-1 (APC) is thought to act positively and synergize with MOM-2 (Wnt) and MOM-5 (Fz). In a phenotype that may be related to *Drosophila* tissue polarity, MOM-2 (Wnt) and MOM-5 (Fz) also regulate spindle orientation through a mechanism that may involve a Gβ protein [60].

studies in the developing *Drosophila* eye support this model and suggest that Fz signals through Dsh to the p21 GTPase Rho A and then to the JNK/SAP kinase bsk (Scheme 2B). This pathway has an intriguing similarity to the pathways proposed to operate during yeast pheromone signalling and the activation of serum response factor (SRF) in mammals [48].

Smoothened

The smoothened receptor (Smo) is a *Drosophila* frizzled homologue that shares 17% homology with Fz compared with 42% amino acid similarity between Fz and Dfz2 [52,53]. Importantly, however, the smoothened–Fz region of homology includes the 'Wnt binding domain', raising the possibility that smo may bind Wg. Although genetic studies argued against a role for smo in Wg signalling during ventral epidermal development, the existing data do not exclude a role for Wg–smo interactions during an early phase of Wg autoregulation [54–56].

Wnt signalling in *Caenorhabditis elegans*

Wnt signalling has also been shown to regulate asymmetric cell divisions in *C. elegans*. Mutations in lin-44 (Wnt) result in the reversal of B- and T-cell polarity in a way that is consistent with the lin-17 (Fz) product functioning as a receptor [57,58]. The *C. elegans* mom-2 (Wnt), mom-5 (Fz), wrm-1 (β-catenin) and pop-1 (TCF) gene products function in what conforms to a consensus Wnt signalling pathway during endoderm development [59–61]. However, mom-2 (Wnt) and mom-5 (Fz), but not wrm-1(β-catenin) and pop-1 (TCF), were required for the correct orientation of mitotic spindles in some early blastomeres [60]. This differential requirement for Wnt signalling components may reflect the fact that the orientation of spindles in early embryos does not require transcription (Scheme 2C).

Mechanism of signal transmission

Little is known about the mechanism by which Wnt binding to Frizzled transmits a signal through to the intracellular surface of Frizzled. Most analysis of ligand binding to 7TMS receptors has concerned small molecule binding to the extracellular loops and membrane-spanning domains [62]. By contrast, Frizzled joins a class of 7TMS receptors, including the lutropin receptor and a calcium-ion receptor that contain large extracellular ligand-binding domains [63,64]. As most 'Wnt binding domains' are linked to their 7TM regions through a region that is predicted to be flexible, the binding of Wnts to the extracellular domain could cause the Frizzled domain to move through large molecular distances, possibly making or breaking contacts with the transmembrane sequences as part of signal transmission.

FRIZZLED TO DISHEVELLED

As the intracellular domains of Frizzled receptors have no enzymic motifs, the Wnt signal is probably transmitted by the recruitment or release of intracellular signalling molecules. Candidate molecules which could interact with Frizzled include Dishevelled and/or heterotrimeric G-proteins. Members of the Dishevelled gene family (Table 1b) are cytoplasmic proteins that probably function as molecular adaptors, since they contain putative protein–protein-interaction domains, but no enzymic motifs (Figure 1). The prototype of the family, Dishevelled (Dsh), was isolated in *Drosophila* and was shown to function downstream of Wg.

Frizzled intracellular coupling

G-protein?

Heterotrimeric G-proteins couple numerous 7TMS receptors to a range of intracellular signalling pathways, including those regulated by phosphoinositide and cAMP second messengers [62]. Data linking Fz to G-protein signalling are currently indirect. Fz regulation of spindle orientation in *C. elegans* blastomeres was suggested to involve a gene encoding a Gβ subunit, and common targets of 7TMS signalling were targeted by Wnt signalling. Firstly, Ca^{2+} transients were induced by Xwnt-5A, in zebrafish embryos, suggesting that phospholipase C was activated by signalling from a Frizzled receptor. Secondly, protein kinase C (PKC) activity was shown to be required for Wg regulation of GSK-3 kinase activity in murine 10T1/2 fibroblasts [65,66].

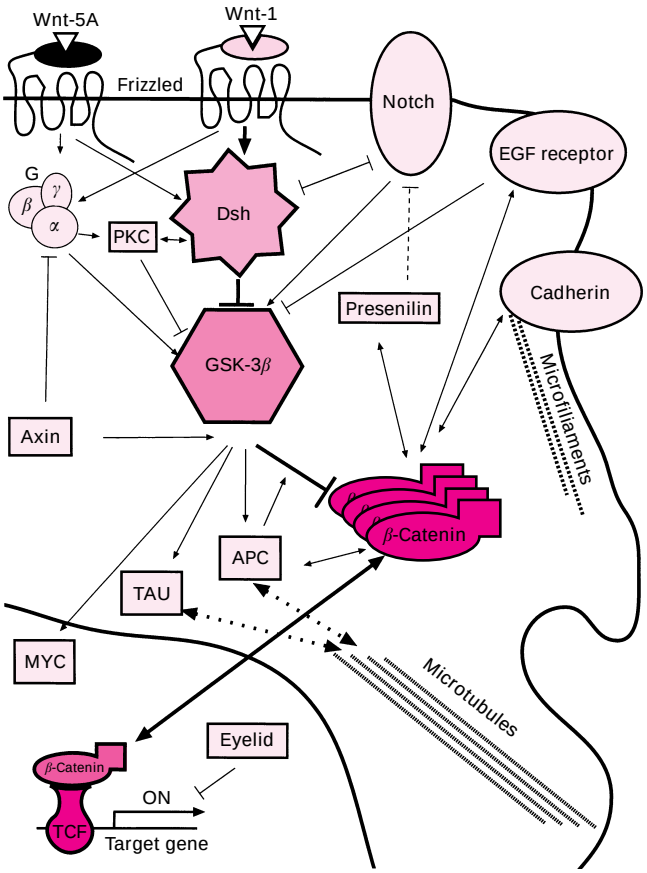
At present it is not clear whether PKC and Ca^{2+} signalling can be integrated into the canonical Wnt signalling model (see Scheme 3). However, recent studies showed that the morphogenic changes induced by Xwnt-5A expression in *Xenopus* embryos were mimicked by the ectopic expression of the 7TMS serotonin (= 5-hydroxytryptamine) receptor, which activated the phosphoinositide (PI) cycle and Ca^{2+} release [8,66,67]. In addition to regulating morphogenesis, activation of the serotonin receptor also mimicked Xwnt-5A function by antagonizing the axis-duplicating activity of Xwnt-8, suggesting that Wnt–Wnt interference may occur downstream of receptor binding [24,67].

Dsh

Genetic analysis of *Drosophila* ventral epidermal development first identified a role for Dishevelled (Dsh) in Wg signalling [51,68]. Genetic epistasis placed Dsh downstream of Wg and upstream of the kinase zw-3. In studies of tissue polarity, Fz was genetically placed upstream of Dsh (Schemes 2A and 2B; [50]). Members of the Dishevelled family encode cytoplasmic proteins with no known enzymic functions [51,68]. Three mouse Dsh homologues (Dvl-1, Dvl-2 and Dvl-3) were identified with overlapping patterns of expression, suggesting redundancy in function [69,70]: a supposition supported by the observation that Dvl-1 null mutant mice were structurally normal, although they had reduced social interactions [71]. Sequence comparisons identified three regions of homology: an N-terminal Dsh homology domain, a central region containing a basic and a PDZ domain, and a DEP domain upstream of the non-conserved C-terminal sequence (see Figure 1).

Dsh homology domain. A 51-amino-acid region from the N-terminus of Dishevelled family members shares sequence similarity with the C-terminus of Axin, a newly discovered intracellular protein coded by the *fused* gene [72]. While the function of this domain is unknown, the expression of Axin in *Xenopus* embryos was shown to interfere with the formation of the endogenous dorsal axis and to prevent the formation of ectopic axes by Xwnt-8 [72]. Further studies showed Axin interfered with Wnt signalling downstream of GSK-3 and that a separate RGS domain (**R**egulators of **G**-protein **S**ignalling) domain was critical for this function (Scheme 3).

PDZ domain. The PDZ, GLGF or DHR domain is an 80–90-amino-acid motif found in over 50 proteins, including PSD-95, ZO-1 and the product of the *Drosophila* tumour suppressor gene *discs large*, *dlgA*. Structural analyses showed that PDZ domains from Dlg and PSD-95 were able to bind four-residue C-terminal peptides with the motif X-(S/T)-X-V (reviewed in [73]). PDZ domains can also form heterotypic dimers with other PDZ domains, and at least one domain has been shown to bind to an



Scheme 3 Potential complexity within Wnt signalling

Differential responses to Wnt family members and Wnt interactions with other signalling pathways may occur at multiple levels of the signalling pathway.

(S/T)XV motif positioned nine residues from the C-terminus [74]. The five PDZ domains from the *Drosophila inaD* gene have been shown to recruit distinct signalling molecules, including PKC and phospholipase Cβ into a highly organized signalling unit with InaD behaving as a molecular scaffold [75]. The PDZ domain of Dsh family members has been suggested to play a role in signal reception from Frizzled receptors, some of which have C-terminal (S/T)-X-V motifs. The replacement of the C-terminal eight amino acids from the *C. elegans* Lin17 frizzled protein with green fluorescent protein (GFP) failed to interfere with Lin17 function *in vivo*, suggesting that the Lin-17's C-terminal motif is not required for signalling [58]. However, this result may not be unexpected, as the Lin-17 Cl-terminal sequence does not fit the PDZ binding consensus. Dsh PDZ domains were shown to be essential for downstream signalling in *Xenopus* embryos and in *Drosophila* cell culture (see the next section).

DEP domain. The DEP domain is a recently identified motif of approx. 80 amino acids that was found in proteins, including pleckstrin and the guanine nucleotide dissociation and GTPase-activating protein families. The DEP domain has been suggested to function in recruiting downstream targets for G-protein-coupled pathways [76]. Little is known about the role of the DEP domain in receiving Wnt signals, but a C-terminal deletion of *Drosophila* Dsh that includes the DEP domain did not prevent downstream stabilization of the Arm protein following over-expression in Cl-8 cells [77].

Frizzled regulation of Dsh

Stimulation of *Drosophila* Cl-8 cells with soluble Wg leads to the accumulation of Arm protein and the membrane translocation of a fraction of Dsh [77,78]. Changes in the compartmental localization of mammalian Dvl-1 and Dvl-2 were also observed following Wnt-1 expression in PC12 cells [79], leading to the suggestion that the membrane fraction of Dsh was active in Wnt signalling [77]. Frizzled may be required for the membrane translocation of Dsh, as Xdsh associated with the cellular membrane when co-expressed with Rfz1 in *Xenopus* animal caps [6]. The mechanism by which signals are transmitted from Wnt, through Fz, to Dsh family members is unclear. For example, it is not known if Fz and Dsh directly interact. However, a plausible model is that Wnt-dependent changes in Frizzled structure increase Dsh affinity and thus Dsh relocalization to the cellular membrane. Once at the cellular membrane, Dsh could then couple to novel sets of downstream factors.

In *Drosophila* Cl-8 cells, the fraction of Dsh protein that translocated to the membrane following Wg treatment was found to be hyperphosphorylated [77]. A Dsh-associated kinase that phosphorylated Dsh was subsequently identified as casein kinase 2 (CK2) [80]. CK2 phosphorylated Dsh within a central region containing the basic and PDZ domains. Dsh phosphorylation was further linked to Wnt signalling by the demonstration that Dfz2 overexpression in *Drosophila* S2 cells resulted in Dsh hyperphosphorylation.

However, Dfz2 overexpression in *Drosophila* S2 cells in the absence of Wg failed to stabilize levels of downstream Arm protein [80]. This finding was unexpected, as Dsh overexpression in S2 cells was associated with hyperphosphorylation and Arm stabilization [77], while ectopic Rfz1 expression in *Xenopus* was sufficient to activate Wnt-target genes [6]. As overexpression of Dsh and Frizzled components can artificially overcome the requirement for Wnt ligands, the finding that Dfz2 over-expression was insufficient suggests that there may be additional Wnt-dependent signals that are activated during ligand binding. While CK2 may phosphorylate Dsh as part of Wnt signalling, it is not yet clear whether the Dsh phosphorylation is required for, or is simply a consequence of, Wg signalling.

Dsh–Notch interactions

The demonstration that the C-terminus of Notch binds to the N-terminal half of Dishevelled suggested an additional mechanism (to Wg–Notch binding) by which Notch and Wg signals may interact [47]. Genetic analyses of wing bristle and margin formation suggested that Wg (through Dsh) inhibited Notch signalling [45–47], while increasing the *notch* gene dosage was shown to reduce the action of dsh, suggesting that Notch may also interfere with Wg signalling. However, in other systems, Notch and Wg signals appear not to interact or have been shown to synergize (reviewed in [81]). This raises the possibility that Notch may transduce a subset of Wnt signals in systems where activation of the canonical pathway is insufficient or is not required for Wnt signalling [54,80].

DISHEVELLED TO GSK-3

Glycogen synthase kinase-3 (GSK-3) is a cytoplasmic serine/threonine kinase that was originally identified from its role in glycogen metabolism. Later studies showed the GSK-3β isoform was functionally homologous with the *Drosophila zeste-white3* (*zw3*) gene product [82]. Genetic studies of *zw3* showed that it functioned downstream of *dsh* and was probably inactivated by

Wg signalling. Little is known about the biochemical pathway leading from Dishevelled activation to inhibition of GSK-3, and it is probable that additional intermediate components remain to be identified. Important and as yet unanswered questions surround the mechanism by which Wnt specificity is maintained while signalling through GSK-3, since the kinase is inactivated by peptide ligands, including insulin and EGF, and has been genetically placed downstream of Notch in signalling by the Delta ligand [83].

Downstream signalling by Dsh

Overexpression of *dsh* and *Xdsh* was shown to activate Wnt signalling (in the absence of ligand) as measured by Arm stabilization in *Drosophila* Cl-8 cells or axis duplication in *Xenopus* embryos [77,84,85]. A deletion analysis of Dsh showed that the PDZ domain and the conserved N-terminus were required for Arm accumulation [77]. Deletion of the PDZ domain from Xdsh resulted in a dominant negative Xdsh that was capable of inhibiting *Xenopus* axis duplication by ectopic Xwnt-8. These assays strongly suggest that the PDZ domain is involved in downstream signalling, although it is not yet clear whether the PDZ domain directly interacts with downstream effectors such as adenomatous polyposis coli (APC)/GSK-3.

Links to GSK-3

Genetic analysis of *Drosophila* ventral epidermal development identified a role for Zw3 in Wg signalling and showed that the mammalian GSK-3β isoform can partially compensate for the loss of Zw3 during *Drosophila* embryogenesis [83,86]. Genetic epistasis showed that Zw3 functioned downstream of Dsh (Table 1b; [87,88]), and recent studies have shown that Dishevelled (Dvl-1) overexpression inhibits GSK-3 activity, as measured by the GSK-3-mediated phosphorylation of the tau microtubule-binding protein [89].

Embryonic expression of *Xenopus* GSK-3 (XGSK-3) containing an inactivating mutation in the kinase active site (dnXGSK-3) induced an ectopic axis in *Xenopus* embryos that closely resembled phenotypes observed following Xwnt-8 and Xdsh overexpression, suggesting that all three factors function in a common pathway [90–92]. As yet it is not yet clear whether dnXGSK-3 functioned by sequestering upstream regulators or downstream effectors of GSK-3 function. Of possible relevance to the mechanism of dnGSK-3 action was the finding that a kinase-dead GSK-3β lost its ability to bind to APC, a known substrate, in colon-cancer cells [93]. By contrast with dnXGSK-3, wild-type XGSK-3 was able to suppress ectopic axes induced by Xwnt-8 and Xdsh [90–92,94], suggesting that GSK-3's normal function is to suppress Wnt signalling mediated by Xdsh.

The consequences of Xwnt-8 expression in *Xenopus* have striking parallels with the phenotypic effects of Li^+ treatment, which can induce a secondary body axis on the ventral side of the embryo and can rescue UV-ventralized embryos [95–97]. Li^+, like Wnt, stabilized Arm/β-catenin by directly inhibiting GSK-3 activity, a mechanism that is distinct from Li^+'s effects on inositol monophosphatase [67,96,98,99].

Mechanisms of GSK-3 regulation

Mammalian GSK-3 has been implicated in signal transduction by growth factors, including insulin [100], insulin-like growth factor (IGF-1; [101]), serum [102], epidermal growth factor (EGF; [103,104]) and Wg [65]. In many cases, including Wg, the inhibition of GSK-3 was reversed by incubation with serine/threonine protein phosphatases, suggesting that GSK-3 was regulated by inhibitory kinases. Studies with inhibitors of PKC suggested that a PMA-sensitive PKC(s) lay upstream of GSK-3 in Wg signalling, and recent observations have shown that Wg induces the translocation of some PKC isoforms to cellular membranes (Y. Patel, T. Dale and M. Fry, unpublished work). As PKC can phosphorylate GSK-3 *in vitro* [105], this raises the possibility that PKC could directly phosphorylate and inhibit GSK-3 activity in response to Wnt signalling. The role of PKC in Wnt signalling in other systems is unclear; however, PKC activity in *Xenopus* embryos peaks during neuralization, a stage at which dnXdsh blocks normal *Xenopus* embryogenesis [106–108].

By analogy with GSK-3 regulation by 7TMS receptors in the slime mould *Dictyostelium*, it was recently suggested that GSK-3 activity may be differentially regulated by Wnt-5A and Wnt-1 class factors [24]. Analyses of GSK-3 regulation by ligands such as insulin have shown that kinase activity can be inhibited by phosphorylation of Ser^9 and that kinase activity requires tyrosine phosphorylation at Tyr^{216} [100,109–113]. Regulation at both these sites provide potential routes for Wnt inhibition of GSK-3 activity; however, no data are yet available on the sites of GSK-3 that are regulated in response to Wnt signalling. However, overexpression of a $Tyr^{216} \rightarrow Phe^{216}$ mutant of GSK-3 did not interfere with wild-type GSK-3 function, as measured by the induction of ectodermal abnormalities in *Xenopus* embryos [94].

GSK-3 TO β-CATENIN

β-Catenin is a member of a multigene family of proteins characterized by the presence of 'Arm' amino acid repeats that mediate a range of protein–protein interactions (Figure 1; Table 1b). Genetic analysis of the *Drosophila* homologue Armadillo (Arm), showed it functioned downstream of GSK-3/zw3 [88, 114,15]. Considerable evidence suggests that the Wnt inhibition of GSK-3 activity prevents the turnover of β-catenin, leading to its accumulation. There is some disagreement whether GSK-3 normally promotes β-catenin turnover by direct phosphorylation or whether additional factors are involved.

Links from GSK-3 to β-catenin

Wnt expression stabilized levels of β-catenin and Arm in mammalian cell-culture models and in *Drosophila*/*Xenopus* embryos [114,116–118]. Loss of Zw3 or the use of dnGSK-3 similarly stabilized β-catenin and Arm levels in both *Drosophila* and *Xenopus* embryogenesis, suggesting that GSK-3 normally functioned to lower β-catenin levels [114,118,119]. Several lines of evidence point to a role for β-catenin phosphorylation in its turnover. The loss of Zw-3 function or the use of dnGSK-3 reduced Arm and β-catenin phosphorylation [115,119], whereas wild-type XGSK-3 phosphorylated β-catenin *in vitro* at a conserved N-terminal site that was also phosphorylated *in vivo* [119]. β-Catenin mutants lacking the phosphorylated sites were more stable in the presence of XGSK-3 [119], suggesting that XGSK-3 phosphorylation of these sites promoted β-catenin turnover. Mammalian β-catenin and *Drosophila* Arm were also stabilized by mutation or deletion of putative GSK-3 target sites [120–123]. However, an unresolved question is why mammalian GSK-3 did not directly phosphorylate β-catenin *in vitro* [93,96].

The mechanism by which β-catenin phosphorylation results in its degradation is not known. However, the N-terminus of β-

catenin/Arm shows a weak match to sequences in the transcriptional inhibitor I$\kappa\beta$a that mediate phosphorylation-dependent ubiquitination and proteolysis [123–125], and recent results have shown that β-catenin is degraded through an ubiquitin-mediated pathway [126]. A possible cofactor role for APC in β-catenin turnover has been suggested [127] (see below).

GSK-3 specificity

As described below, increased β-catenin levels were sufficient to produce Wnt-like phenotypes, suggesting that β-catenin may be the key GSK-3 target for Wnt signalling. However, GSK-3 has the potential to regulate a number of pathways, as it can phosphorylate targets including glycogen synthase, several transcription factors (c-Myc, c-Jun, c-Myb and NfATC), the microtubule-associated tau proteins and APC [82,98,128]. As yet it is not clear whether Wnt signalling alters the phosphorylation of these GSK-3 targets or whether the physiological consequences of regulating these GSK-3 targets would be observed in the 'standard' Wnt assays described below.

Conversely, the range of GSK-3 regulators raises the question as to how Wnts maintain specificity while signalling through a common kinase. One theory, based on popular current models, is that GSK-3 could be recruited into a highly organized signalling 'transducisome' complex by the action of scaffolding protein (for an example, see [75]). In this context, the finding that APC can bind both β-catenin and GSK-3 suggested that APC may function as a signalling scaffold (see below).

β-Catenin

β-Catenin encodes a 90 kDa protein with several structural domains (Figure 1) [129,130]. The N-terminus contains the XGSK-3 phosphorylation sites followed by a region that mediates binding to α-catenin. The central domain of the protein contains 13 imperfect 'Arm' repeats that fold to make a superhelix containing a positively charged groove that is hypothesized to interact with acidic regions of APC, TCF transcription factors and cadherin cell-adhesion molecules [131]. Binding of APC and cadherins to β-catenin is mutually exclusive [132,133], but it is not yet clear whether TCF binding interferes with APC or cadherin binding [130,133–135]. β-Catenin complexes containing TCF and cadherins were found in the nucleus and membrane respectively, whereas complexes containing APC were found in the cytosol and at the tips of plasma-membrane protrusions [120,136,137] (reviewed in [129]).

Several additional proteins are included within the 'Arm family' on the basis of the presence of 42-amino-acid 'Arm repeats' [138,139]. Two members in particular have been implicated in Wnt signalling. Plakoglobin (γ-catenin) was stabilized by Wnt-1 expression in mammalian cell culture, and induced *Xenopus* axis duplication when overexpressed [117,140–142]. However, unlike β-catenin, plakoglobin was not required for the formation of the endogenous axis in *Xenopus* [141,143]. Secondly, APC, which interacts with β-catenin (see below), contains a set of N-terminal Arm repeats, although it is not clear what function the repeats have in APC function (Figure 1; [144]).

β-Catenin stabilization

The raised levels of β-catenin resulting from Wnt signalling were necessary and sufficient for axis formation in *Xenopus* and zebrafish embryos as shown by ectopic expression or depletion of β-catenin (reviewed in [129]). However, Wnt signalling may not be the only mechanism by which soluble levels of β-catenin are regulated. In some cell types, tyrosine phosphorylation of β-catenin was associated with *ras* or *src* transformation and EGF treatment, leading to the disruption of adherens junctions and the accumulation of tyrosine-phosphorylated β-catenin [145–147]. Accumulation of β-catenin was also reported following the induction of c-*jun* expression or retinoid treatment in mammary epithelial cells [148,149]. Most significantly, recent data suggest that endogenous *Xenopus* axis formation (see Table 1a) may not require Wnt ligands, as microtubule-mediated transport may asymmetrically relocalize β-catenin and thus generate a 'Wnt-like' signalling centre without the requirement for upstream Wnt signalling components [118,150–152].

β-Catenin-binding partners

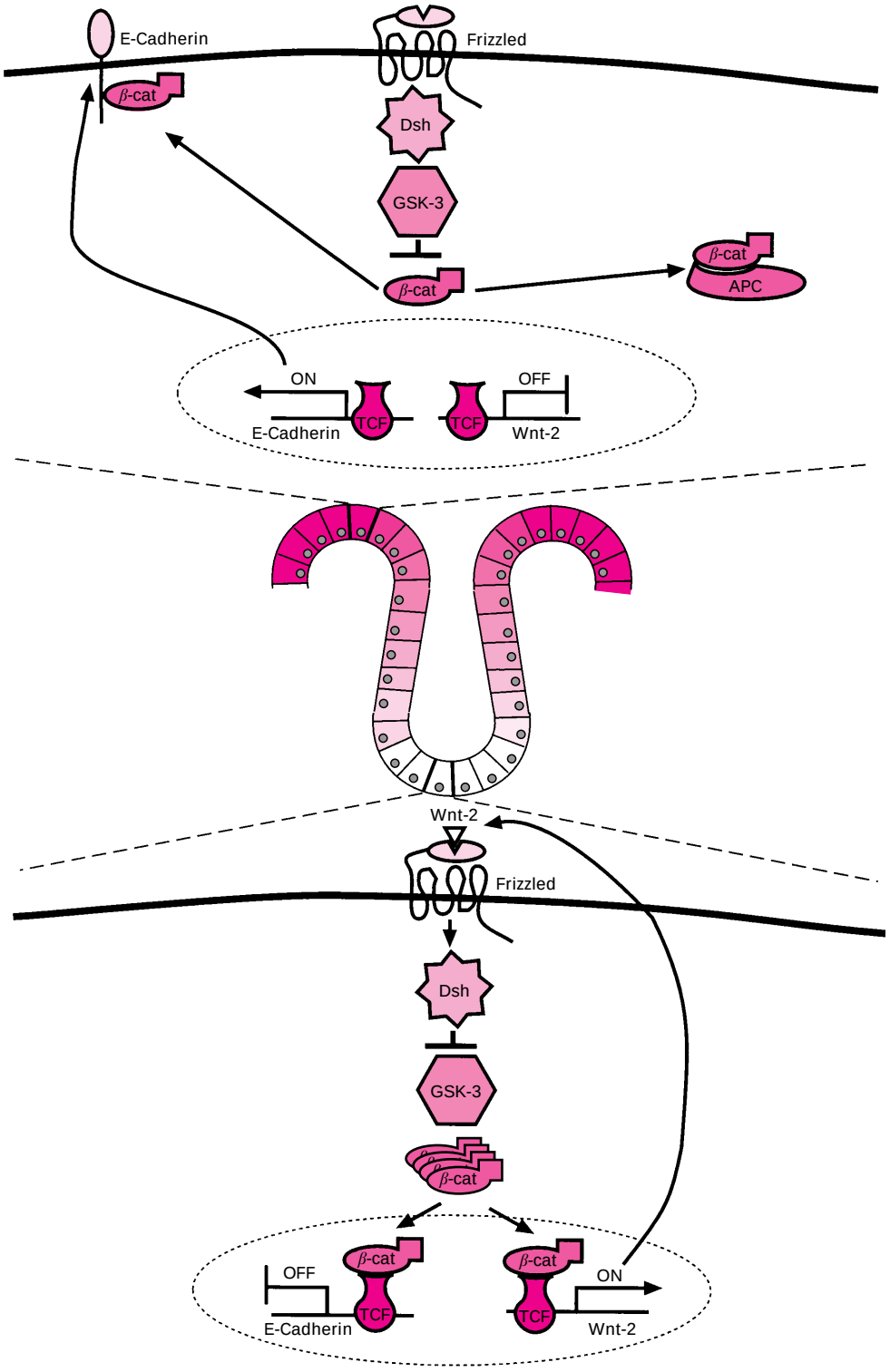
As described below (see also [153]), most genetic evidence points to a central role for TCF factors in mediating Wnt-regulated transcription. However, β-catenin can interact with a range of additional proteins. The functions of β-catenin binding to cadherins and APC are discussed below; however, catenins have also been show to interact with the EGF receptor and the Alzheimer's-disease gene *presenilin*, suggesting routes by which Wnt signalling may regulate disparate molecular targets [146, 154,155].

Cadherins. Cadherins are Ca^{2+}-dependent adhesion molecules that mediate cell–cell interactions at adhesive junctions (reviewed in [129,156]). The intracellular domain of cadherins interact with the cytoplasmic adaptor proteins β-catenin or plakoglobin. While complexed to cadherins, β-catenin is also able to interact with α-catenin, a cytoplasmic protein with similarity to vinculin, which links actin filaments to the adherens junctions.

By focusing on the targets of the canonical Wnt signalling pathway, mutational analyses have shown that regions of β-catenin required for Wnt signalling are separable from those required for cell adhesion, suggesting that the cadherins are not required for Wnt signalling [130,157,158]. However, changes to cadherin expression can affect Wnt signalling, while Wnt signalling can influence cell adhesion [140,159], possibly by changing levels of free β-catenin and thus indirectly altering the abundance of β-catenin-containing cadherin and TCF complexes.

Levels of cadherin expression affected free β-catenin pools and signalling, in *Xenopus* embryos, ES cells and *Drosophila* embryos [8,157,158,160,161]. However, as E-cadherin-deficient L-cells were able to respond to Wnt-1 by stabilizing β-catenin, it is unlikely that the reductions in E-cadherin expression were sufficient or were required for Wnt signalling [162]. Cell adhesion may be a downstream target of Wnt signalling as the adhesive functions of cadherins were regulated by interactions with β-catenin [163,164]. Wnt-1 overexpression in several cell lines stabilized cadherin/β-catenin interactions and increased cell adhesion [116,117,140,147], while Xwnt-5A and dnXdsh induced morphogenetic movements in *Xenopus* embryos that probably involved changes in cell adhesion [8,106]. However, the relationship between soluble and cadherin-bound β-catenin may not be a simple equilibrium, as each β-catenin pool was differently phosphorylated [115,123] and different phenotypes were observed when Arm/β-catenin levels were partially depleted (Wnt-mutant phenotypes) compared with fully removed (cell-adhesion effects) [160,165,166].

APC. Mutations in the APC tumour-suppressor gene have been linked to inherited and sporadic cancers of the colon [127,144,167,168]. APC encodes a 300 kDa multifunctional protein with several structural domains (see Figure 1 for structure).



Scheme 4 Model for APC function in the colon

This model proposes a role for APC downstream of β-catenin in the regulation of Wnt-dependent transcription (GSK-3 ⊣ β-catenin → APC). At the base of the colonic crypt, APC is absent and Wnt signalling occurs through the canonical Wnt signalling pathway (see Scheme 1). Wnt signalling is proposed to directly or indirectly regulate Wnt gene expression, generating an autocrine signalling loop. In differentiating colonic epithelial cells, APC expression (graded pink/red tinting) titrates β-catenin levels and interferes with the Wnt autocrine loop. E-cadherin may further titrate β-catenin levels, since the E-cadherin promoter contains TCF-binding sites and could be a target of Wnt signalling [163]. The three main supporting observations for the GSK-3 ⊣ β-catenin → APC order of function are as follows. First, APC is probably not required for Wnt signalling, as it was not expressed in enterocytes in the lower third of the colonic crypts [206], and dAPC was not required for Wg signalling in *Drosophila* [182]. Secondly, the only regions required for β-catenin turnover were APC's β-catenin-binding repeats [175], as would be predicted if APC functioned by titering β-catenin from TCF complexes. Thirdly, Wnt-2 expression was found in all colorectal tumour samples at all Dukes' stages of progression [207], as would be expected if it functioned in an autocrine loop.

The N-terminus contains an oligomerization domain followed by seven repeats of an 'Arm' motif. Toward the centre of the molecule are two sets of related repeats, three 15-amino-acid repeats followed by seven 20-amino-acid repeats, both of which can bind β-catenin [133,169,170]. Following the 20-amino-acid repeats is a basic region, while the C-terminus has an S/TXV motif that possibly interacts with the PDZ-containing human homologue of Dlg, the product of a *Drosophila* tumour suppressor gene [171]. The C-terminal third of APC has been shown to mediate interactions with microtubules and a novel protein EB1 [172–174].

Links between APC and Wnt signalling. APC regulates levels of β-catenin in colon-cancer cell lines and has been suggested to be involved in Wnt signalling, such that a loss of APC function is equivalent to a positive Wnt signal. However, there is great uncertainty whether endogenous APC is part of the Wnt signalling pathway, whether it functions in parallel with the Wnt pathway or whether it is simply a target of Wnt signalling downstream of β-catenin.

The first suggestion of a link between APC and Wnt signalling was the finding that β-catenin physically interacted with APC and that the most common mutations of APC that led to colon cancer resulted in the loss of β-catenin-binding sites [169,170]. The introduction of wild-type APC (or a region containing the β-catenin-binding repeats) into cell lines containing mutant APC reduced cytoplasmic β-catenin levels, suggesting that the APC–β-catenin interaction was important for the regulation of β-catenin levels [175]. Further links to Wnt signalling came from the finding that complexes containing APC and β-catenin bind GSK-3, and that GSK-3 can phosphorylate and enhance β-catenin binding to the 20-amino-acid repeats [93]. Finally, cells containing high levels of β-catenin and mutant APC were shown to drive constitutively high levels of transcription from a TCF-dependent promoter, while introduction of APC suppressed both β-catenin levels and transcription [122,176].

As increases in cytoplasmic β-catenin levels are a key intermediate in Wnt signalling, it was proposed that APC functioned between GSK-3 and β-catenin to down-regulate β-catenin levels in response to the activity of GSK-3 [168,177]. In this role, APC has been suggested to function as a molecular scaffold, coordinating the actions of GSK-3 and β-catenin. Alternatively, APC's role in β-catenin turnover has been suggested to be that of a ubiquitinylation co-factor for the targeted degradation of β-catenin [126].

The importance of the model placing APC function between GSK-3 and APC was that it suggested a mechanism for APC's role in colon tumorigenesis. The loss of APC function was proposed to reduce β-catenin turnover, thereby generating a signal equivalent to that of the Wnt-1 mammary oncogene [178–181]. Support for this model was provided by the finding that β-catenin was mutated in some melanoma and colon-cancer cell lines [121,122,168,176]. The oncogenic mutations in β-catenin substituted N-terminal GSK-3 phosphorylation sites that were critical for β-catenin stability in *Xenopus* [119]. As would have been predicted, these mutations increased β-catenin stability and led to increased transcription from TCF-dependent reporter plasmids [122,176]. The importance of controlling β-catenin levels in preventing colon tumorigenesis was emphasized by the finding that colon-cancer cell lines either had an APC mutation or had a β-catenin mutation, supporting the mechanistic contention that both factor functioned in the same pathway [168]. The GSK-3 → APC ⊣ β-catenin model for APC's role in Wnt signalling was recently brought into question by the surprising finding that *Drosophila* APC (D-APC) was not required for Wg function in ventral epidermal development [182]. However, these studies were not conclusive, as signalling by residual levels of maternal D-APC could not be excluded.

A second prediction from the GSK-3 → APC β-catenin degradation model is that overexpression of APC in *Xenopus* embryos should block Wnt signalling by increasing the turnover of β-catenin and/or by binding free β-catenin. Unexpectedly, however, overexpression of *Xenopus* APC (or overexpression of the APC β-catenin-binding repeats) positively induced *Xenopus* axis duplication and failed to change β-catenin levels [183]. A positive signalling role for APC upstream of β-catenin and in parallel to Wnt and Fz was supported by recent studies of the *C. elegans* APC gene, APR-1, during gut induction [59,60]. It is thus probable that the role of APC is highly dependent on the context in which it is expressed. Most of the data that hypothesizes a role for APC in the turnover of β-catenin are derived from studies of colon-cancer cell lines. As discussed below, it is possible to reinterpret this data in a model that excludes a direct role for APC in β-catenin turnover.

A model for the function of APC in the colonic epithelium. As shown in Scheme 4, APC could function downstream of β-catenin to interfere with Wnt autocrine signalling during enterocyte differentiation. The key proposal in this model is that APC breaks a Wnt autocrine signalling loop by titering β-catenin away from transcriptionally active TCF. Autocrine Wnt signalling has also been shown during early stages of *Drosophila* ventral epidermal development, where Wg regulates its own expression [54,184]. Some predictions from this model are similar to the previous (GSK-3 → APC ⊣ β-catenin) model, in that the loss of APC function or the mutation of β-catenin should activate Wnt-dependent transcription in colorectal cancers [122]. Other predictions that distinguish between the models can be easily tested.

In addition to interfering with autocrine Wnt signalling, APC may target a range of alternative functions, including the induction of apoptosis, cyclin function/cell cycle and cell motility/microtubule organization (reviewed in [144]). Many of these functions may be regulated by Wnt function through the association with β-catenin. For example, APC associated with β-catenin was found in clusters at the tips of plasma-membrane protrusions [120], where it may control morphogenetic movements such as tubulogenesis [137].

β-CATENIN TO TCF

A very direct link in Wnt signalling occurs between the accumulation of β-catenin and the regulation of transcription, as β-catenin has been shown to bind TCF DNA binding factors (Figure 1) and to directly regulate transcription.

Links from β-catenin to TCF factors

Screens for proteins that interacted with β-catenin identified members of the TCF family (XTCF-3 and LEF-1) ([163,185,186]; reviewed in [153]). Proteins from the TCF family bind DNA and contain a high-mobility-group (HMG) domain. Murine family members include LEF-1 (lymphocyte enhancer binding factor 1) and TCF-1, which have functions in lymphopoiesis and epithelial mesenchymal interactions respectively [187,188]. Less-well-characterized members include TCF-3 and TCF-4 [189]. Screens of *Xenopus* have shown the existence of three isoforms of XTCF3 [185], while family members have also been detected in *Drosophila* (pangolin) and *C. elegans* (pop-1) [190,191], suggesting the gene family is highly conserved.

Genetic epistatic analysis of *Drosophila* TCF (dTCF or pangolin) showed that dTCF was required downstream of Arm during

Wg signalling in the ventral epidermis [135,191]. A variety of phenotypes resulting from the loss of dTCF were similar to that of Wg mutants, suggesting that dTCF functions as a transcriptional activator rather than as a repressor, since the loss of a repressor would be expected to generate a constitutively active Wg phenotype. As expected, overexpression of LEF-1 in *Drosophila* using a heat-shock promoter generated a positive Wnt phenotype similar to that produced by Wg overexpression [192].

Using *Xenopus* axis duplication as an assay for Wnt signalling, it was shown that LEF-1 synergized with β-catenin in the induction of an ectopic axis [163,186]. By contrast, overexpression of the *Xenopus* TCF family member XTCF-3 failed to induce axis duplication [185]. The response to XTCF-3 was more likely to be physiological than that of LEF-1, as the endogenous XTCF-3 gene was expressed during *Xenopus* embryogenesis and was thus a candidate for establishing the endogenous axis.

Mechanisms of transcriptional regulation

Analyses of domains mediating TCF/β-catenin interactions showed that the conserved N-terminal 60 amino acids of TCF mediated interactions with β-catenin/Arm, while Arm repeats 3–8 of β-catenin/Arm were necessary for binding to TCF [135,185,186]. The importance of TCF–β-catenin interactions for Wnt signalling was shown by studies with TCF factors that lacked the N-terminal β-catenin-binding domain (ΔN-TCF). When injected into *Xenopus* embryos, ΔN-XTCF interfered with axis induction by ectopic β-catenin [185] and ΔN-LEF-1 failed to activate axis duplication [186]. Similarly, expression of ΔN-dTCF in *Drosophila* embryogenesis disrupted Wg signalling in the ventral epidermis [135]. Axis formation by LEF-1 was also dependent on the presence of the DNA-binding HMG domain [186].

Deletion analyses of β-catenin showed the C-terminus of β-catenin was required for transcriptional activation from a reporter construct containing multimerized copies of a TCF DNA-binding sequence [135]. The C-terminal region of β-catenin had also been shown to independently activate transcription when fused to a heterologous DNA binding domain [133,135]. Both the C-terminus of Arm and repeats 3–8 of Arm were required for Wg signalling in *Drosophila* [130], suggesting that both Arm–dTCF-interactions and transcriptional activation by Arm were required for Wg signalling.

Simple model of Wnt transcriptional regulation

These observations led to the proposal of a simple model for Wnt-mediated transcriptional activation (Scheme 1). Wnt mediated increases in β-catenin levels raise the concentration of TCF–β-catenin complexes, which then bind to target TCF DNA elements. Once bound, the C-terminus of β-catenin partner activates transcription by interacting with other components of the transcriptional machinery.

Support for this model came from studies showing a LEF-1 DNA-binding element was required for Wg regulation of Ultrabithorax (Ubx) expression in the *Drosophila* midgut [192]. However, Wg transcription in the midgut also required the presence of a neighbouring decapentaplegic (dpp) element, leading to the suggestion that the LEF-1–Arm complex coordinated signalling from the Wg and dpp elements by bending the DNA structure to alter the topology of the transcriptional enhancer [186,192]. Future studies would be expected to identify TCF-binding elements within promoters for *Drosophila* genes, including engrailed, and *Xenopus* genes, including Siamois [193,194]. Oncogenic targets for Wnt signalling should also be identified within the colon.

Another prediction from the model above was that β-catenin (bound to TCF) should accumulate in the nucleus following Wnt signalling. As expected, nuclear β-catenin staining in Neuro2A cells and two-cell mouse embryos required ectopic expression of LEF-1 [163], leading to the suggestion that the nuclear localization signal within TCF mediated the cytoplasm-to-nucleus translocation of the TCF–β-catenin complex. Studies of dorsal development in *Xenopus* and zebrafish embryos showed endogenous β-catenin accumulated in dorsal nuclei, while dnGSK-3 could enhance nuclear accumulation [119,195].

As all the above data were consistent with a strictly nuclear role for β-catenin in transcriptional induction, it was a suprise to find that overexpression of a cytoplasmically tethered plakoglobin molecule induced axis duplication in *Xenopus* with equal efficiency to wild-type plakoglobin [196]. To account for this observation, the authors suggested that plakoglobin (or β-catenin) titrated XTCF-3 in the cytoplasm, thereby relieving a suppressive effect of uncomplexed XTCF-3 on target genes. Studies of *C. elegans* Wnt (MOM-2) signalling have also suggested that transcriptional activation may occur through TCF (POP-1) derepression [59].

Specificity in TCF-dependent transcription

Wnt signalling is known to target different genes at different phases of development. For example, Wg regulates engrailed during ventral epidermal development and dpp during wing development [197,198]. However, little is known about how specific Wnt targets are selected at each stage. In preparation for Wnt signal reception, the chromatin organization of target genes may 'open' to allow the exchange of TCF factors. The expression of particular TCF transcription factors may be required for chromatin reorganization, as has been shown for myogenin, a helix–loop–helix transcription factor, in somitic development [199]. Alternatively, the differential expression of TCF factors may determine which of two otherwise equipotential cells will respond to Wnt signals.

Specificity in Wnt target selection may also result from combinatorial interactions with other spatially restricted ligands. Interactions between the dpp and Wg signalling pathways were integrated by the Ubx promoter [192], while the recently identified eyelid gene (Bright family of DNA-binding proteins) antagonized Wg signalling in multiple tissues and may itself be regulated by other signalling pathways [200]. Specificity through signal integration may also occur at levels above that of DNA binding. As described previously, Notch–Dsh interactions and the regulation of GSK-3/β-catenin by alternative ligands offer plenty of opportunities for signal integration.

FUTURE DIRECTIONS

While some of the canonical elements of the Wnt signalling pathway have been identified, it is too early yet to conclude that a physical signal-transduction chain has been established leading from the cell surface to transcription in the nucleus. Once this link is established, some of the major questions are likely to concern how Wnt signalling targets non-transcriptional targets such as gap-junctional permeability and spindle orientation [60,201]. A second major question will concern how Wnt signalling maintains its specificity while signalling through factors such as GSK-3, which is targeted by other ligand pathways. A related question of specificity concerns the biological logic of having many Wnt ligands, Frizzled receptors, Dsh adaptor proteins, catenins and TCF factors.

The examples of Notch/Dsh inhibition and Wg/dpp synergy will probably be the first among many methods by which Wnt

signalling interacts with other signalling pathways to generate developmental pattern (see the Wnt review in [2]). Finally, the re-use of the Wnt signalling pathway in multiple developmental contexts requires that the 'Wnt switch' is repeatedly reset. As yet little is known about the down-regulation of Wnt signalling. However, this is likely to be a key area of focus, as a failure of down-regulation is a major factor contributing to the oncogenic functions of various Wnt signalling components.

I thank Matt Smalley, Lee Fryer and Mike Fry for their comments on the manuscript before its submission, and to Sally Townsend and Rita Matthews for their help in collating references.

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