Wnt signalling: **Antagonistic Dickkopfs**

Aaron M. Zorn

Dickkopf proteins are secreted antagonists of the Wnt cell signalling molecules, which have a novel mode of action. Dickkopf1 binds to the LRP5/6 Wnt co-receptor and prevents the formation of active Wnt–Frizzled–LRP5/6 receptor complexes, thus blocking the canonical Wnt–β-catenin pathway.

Address: Wellcome/CRC Institute of Cancer and Developmental Biology, Tennis Court Road, Cambridge CB2 1QR, UK.

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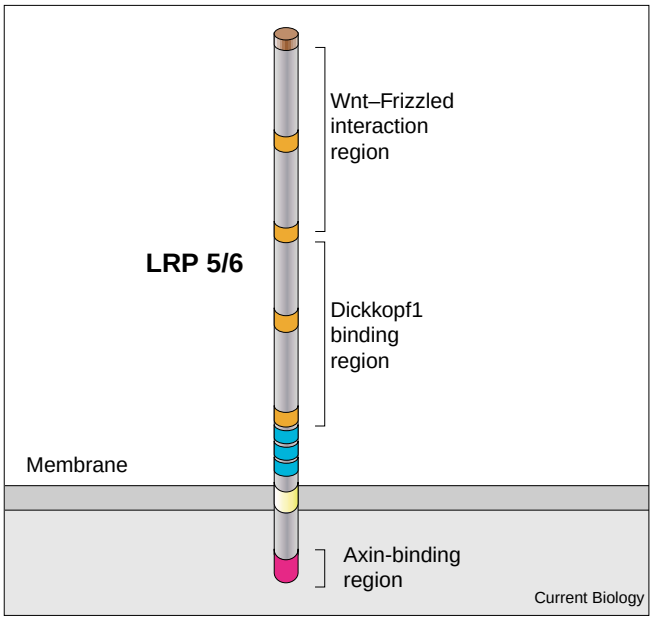
Secreted Wnt glycoproteins are one of the major families of cell signalling molecules and have been implicated in many important processes of metazoan development, as well as in tumour formation [1,2]. Wnt ligands initiate signalling by interacting with two types of receptor molecule at the cell membrane. The Frizzled family of receptors are serpentine seven transmembrane domain proteins that interact with Wnt ligands via a cystiene-rich extracellular domain [1]. Recently a second type of Wnt receptor was discovered, which is related to the low-density lipoprotein (LDL) receptor and is known as LRP5/6, for LDL receptor-related protein. The two closely related proteins LRP5 and LRP6 — the *Drosophila* homologue of which is called Arrow — are single-pass transmembrane proteins which have four extracellular epidermal growth factor (EGF) type repeats and an intracellular tail with potential protein interaction motifs [3–6] (Figure 1). It is currently thought that Wnt must bind both LRP5/6 and Frizzled to form a functional ligand–receptor complex that activates the canonical Wnt–β-catenin pathway [3–6].

In cells not receiving a Wnt signal, a large intracellular complex containing the protein Axin promotes the degradation of the key protein β-catenin [1,2]. In the canonical Wnt pathway, binding of Wnt to its two receptors results in the inactivation of the Axin-containing complex; β-catenin is then no longer degraded and translocates to the nucleus, where it interacts with the TCF family of transcription factors to activate Wnt response genes (Figure 2a,b), which ultimately influence cell fate or behaviour [1,2]. This intracellular Wnt pathway is used over and over again in different contexts throughout the life of an organism. Misregulation of this potent cell signalling pathway can cause developmental defects and tumours [1,2], therefore, the precise regulation of where, when and how much Wnt signalling occurs in a given tissue is critical.

Figure 1

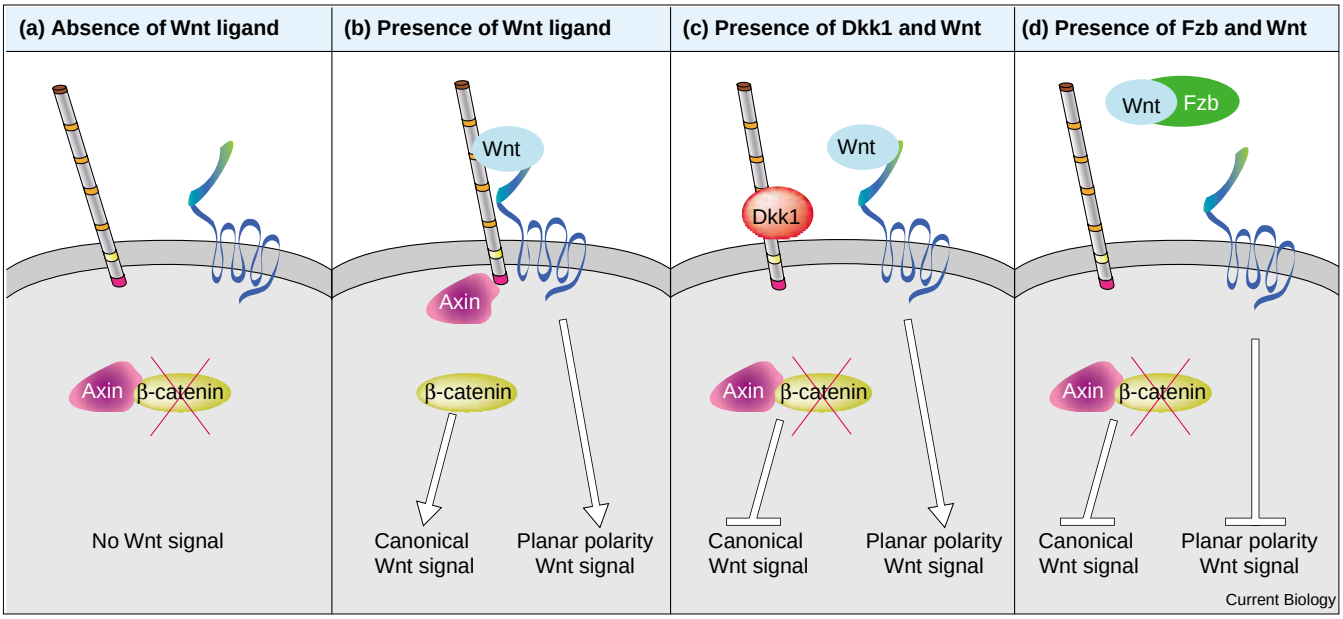


Structure of the LRP5/6 Wnt co-receptor. The diagram shows the general features of two closely related, single pass transmembrane proteins LRP5 and LRP6. The amino-terminal signal sequence (brown) is followed by an extracellular domain containing four epidermal growth factor (EGF)-like repeats (orange) and three LDL-receptor repeats (blue) proximal to the transmembrane domain (yellow). Dkk1 binds to a region encompassing the 3rd and 4th EGF repeats, while the 1st and 2nd EGF repeats are required for LRP5/6 to interact functionally with the Wnt–Frizzled complex [13]. The intracellular domain contains an Axin-binding site (pink) [6].

It has recently become clear that secreted Wnt antagonists play important roles in regulating Wnt signalling. One of the best examples of this is head induction in *Xenopus* embryos, which requires the inhibition of Wnt signals that would otherwise promote the formation of posterior structures [7]. The head-forming region of the *Xenopus* gastrula, known as the Spemann organiser, expresses a number of secreted Wnt antagonists, such as Cerberus [8], Fzb [9] and Dickkopf1 (meaning big head or stubborn) [10]. These Wnt antagonists are believed to repress posterior Wnt signals, thus allowing head development. With the exception of Dickkopfs (Dkks), all of the other known families of secreted Wnt antagonists function by binding extracellular Wnt ligands, thus preventing them from interacting with Frizzled receptors [9,11,12].

The mechanism by which the Dickkopf family of proteins (Dkk1–4 in humans [10]) antagonise Wnt signalling was until recently an enigma. Several new papers [13–15] — one

Figure 2



Regulation of Wnt signalling by Dickkopf and Fzb family Wnt-antagonists. (a) In the absence of a Wnt ligand the axin-containing complex destabilises β-catenin and there is no signal. (b) Wnt ligands bind to both Frizzled and LRP5/6 to form an active receptor complex. LRP5/6 may then recruit and destabilise Axin, contributing to increased β-catenin stability. Cytosolic levels of β-catenin rise and it translocates to the nucleus to activate the canonical Wnt pathway. Wnt–Frizzled complexes can also signal via an alternative, Planar Polarity, Wnt pathway. (c) Dkk1 binding to the LRP5/6 receptor inhibits its interaction with the Wnt–Frizzled complex. Dkk1 may also induce the release of Axin, allowing it to participate in β-catenin degradation. Thus Dkk1 blocks the canonical Wnt pathway, but Wnt–Frizzled complexes can still signal via the Planar Polarity pathway. (d) Wnt-binding antagonists such as Fzb sequester Wnts in the extracellular space preventing activation of both the canonical and the Planar Polarity Wnt pathways.

published recently in *Current Biology* [14] — have now provided compelling evidence that Dkk1 antagonizes Wnt signalling by acting as an inhibitory ligand of the recently discovered LRP5/6 co-receptors. Thus Dkk1 exemplifies a completely new mode of Wnt regulation, which has important implications for the specificity and fine-tuning of Wnt activity during its diverse roles in development.

Dkk1 is an inhibitory ligand of the LRP5/6 Wnt co-receptor

The Niehr's group [10] originally identified Dickkopf1 as a head inducer in *Xenopus*, and their data suggested that Dkk1 blocks cells from receiving the Wnt signal. But how Dkk1 does this was not clear, because Dkk1 could not bind Wnt proteins like the other Wnt antagonists are known to do [13–15]. Furthermore, Dkk1 could not interact with Frizzled, nor could it prevent Wnt from associating with Frizzled receptors [13,14]. Dkk1 therefore blocks Wnt signalling in a manner distinct from other Wnt antagonists. The identification of the Wnt co-receptors, LRP5/6 [3–6] provided a new potential target for Dkk action.

Mao *et al.* [13] and Bafico *et al.* [15] both found that Dkk1 binds readily to cells overexpressing LRP5/6, but not to cells overexpressing Wnt or Frizzled. That Dkk1 and Dkk2 interact directly with the extracellular domain of LRP5/6 was confirmed by co-immunoprecipitation assays [13–15]. All three groups found that Dkk1 binds LRP5/6 with a high affinity, exhibiting a disassociation constant of 0.3–0.5 nM [13–15], consistent with the observation that low concentrations of Dkk1 inhibit Wnt signalling [14,15]. In fact, Dkk binds to LRP6 with an affinity significantly stronger than that reported for interactions between Wnt and Frizzled.

Dkk1 blocks interaction between Wnt–Frizzled and LRP5/6

The discovery that Dkk1 and LRP5/6 physically interact suggested that Dkk1 might somehow inhibit the Wnt–Frizzled–LRP5/6 receptor complex. If Dkk1 represses LRP6, then blocking LRP6 activity by other means should mimic the effects of Dkk1. This is exactly what Mao *et al.* [13] found using a dominant-negative form of LRP6, which mimicked the head-inducing activity of Dkk1 in *Xenopus* embryos. But it remained unclear how Dkk1 binding to LRP5/6 blocks Wnt signalling. Semënoz *et al.* [14] found that even small amounts of Dkk1 blocked the formation of Wnt-induced Frizzled–LRP6 complexes in protein binding assays. To confirm that Dkk1 must bind to LRP5/6 to exert its effects, a single point mutation in Dkk1 was

generated which prevented it from binding to LRP6. As expected, this mutant Dkk1 could not antagonise Wnt signalling and could not block the formation of the Wnt–Frizzled–LRP6 receptor complex [14]. Together, these results suggest that Dkk1 inhibits Wnt signalling by binding to the extracellular domain of LRP5/6 and preventing the formation of active Wnt–Frizzled–LRP5/6 receptor complexes (Figure 2c).

To understand better how Dkk1 regulates LRP6 activity, Mao *et al.* [13] performed a structure–function analysis of LRP6. They found that Dkk1 binds to a region of the LRP6 extracellular domain that is not required for LRP6 to interact functionally with Wnt or Frizzled (Figure 1). This implies that Dkk1 and Wnt might not compete for the same binding site on LRP6, but exactly where Wnt or Frizzled binds remains to be determined. Forms of LRP6 lacking the intracellular domain were found to act as dominant-negative mutants that block Wnt signalling [4,13], while in contrast, an LRP6 mutant lacking the entire extracellular domain behaved as a constitutively active receptor, signalling in the absence of Wnt [6,13]. This suggests that the extracellular domain of LRP6 regulates its signalling activity, and perhaps Dkk1 binding alters the conformation of LRP6.

Another possible mechanism by which Dkk1 might regulate LRP5/6 has recently been suggested by the discovery that the intracellular domain of LRP5 interacts with Axin [6], a negative component of the intracellular transduction pathway. This interaction with Axin is required for LRP5 to potentiate Wnt–Frizzled signals. Wnt signalling appears to stimulate the recruitment of Axin to LRP5, at the membrane, where Axin is degraded. This is accompanied by a stabilization of β-catenin [6]. Thus LRP5/6 may promote Wnt signalling by participating in the inactivation of the Axin-containing complex that destabilizes β-catenin. Dkks might also regulate LRP5/6 activity at this level. In addition to blocking the formation of LRP5/6–Frizzled complexes, perhaps Dkk1 also induces the release of Axin from LRP5, thus allowing Axin to destabilize β-catenin (Figure 2c).

Antagonists may provide specificity to Wnt signalling

One question emerging from this work is why are there different classes of Wnt antagonists? One possibility is that Wnt-binding antagonists, such as Fzb, and the LRP-binding Dkks have qualitatively distinct functions. It turns out that Wnt signalling through Frizzled receptors can also activate an alternative, β-catenin-independent, pathway known as the 'planar polarity' pathway, which regulates cell shape [16]. The *Drosophila* LRP6 mutant *arrow* appears to be deficient in all Wnt/β-catenin signalling, but the planar polarity pathway appears largely intact [3].

Recently it has been shown that cell movements during vertebrate gastrulation rely on this planar polarity pathway [17,18]. Semënoz *et al.* [14] found that a dominant-negative Frizzled receptor could block gastrulation cell movements in *Xenopus* embryonic explants, but Dkk1 and a dominant-negative LPR6 had no effect. Thus LRP6 and Dkk appear to be specific to the canonical Wnt/β-catenin pathway, whereas Frizzled is used in both the canonical and the planar polarity pathways. Dkk may play an important role in determining which of these two Wnt signalling pathways is activated in response to a Wnt signal. Dkk would block the β-catenin catenin pathway but leave the cells still able to respond via the planar polarity pathway. In contrast, Wnt-binding antagonists such as Cerberus or Fzb probably inhibit both the canonical and the planar polarity Wnt pathways (Figure 2).

The identification of LRP5/6 and its inhibitory ligand Dkk1 provide a whole new level of regulation for the Wnt pathway. In many respects this has stimulated more questions than it has answered. Interestingly, not all Dkk proteins function in the same way: Dkk1 and Dkk4 antagonise Wnt signalling, but Dkk2 and Dkk3 do not inhibit Wnt signalling [19,20]. In fact, in some contexts Dkk2 synergises with Wnt signals [12,20]. Is it possible that Dkk2 can act as a positive ligand of LRP5/6 to stimulate Wnt signalling? Thus Wnt regulation outside the cell is becoming increasingly complex. Now the challenge is to determine how different combinations of Wnts, LRPs, Frizzled, Wnt-binding antagonists and Dkks interact to finely tune where, when and how cells respond to Wnt signals.

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