doi:10.1038/nature14208

CELL SIGNALLING

Disarming Wnt

The secreted enzyme Notum has been found to inhibit the Wnt signalling pathway through removal of a lipid that is linked to the Wnt protein and that is required for activation of Wnt receptor proteins.

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Cells signal to one other through secreted molecules that are conserved across the evolutionary spectrum. One class of these signals is Wnt proteins, which influence the balance between proliferation and differentiation in many cell types, including stem cells[1]. Because this balance is crucial for normal tissue maintenance, and overactivation of Wnt signalling can cause cancer, the activity of Wnt signals is tightly controlled by various extracellular molecules. In a paper published on *Nature*'s website today, Kakugawa *et al.*[2] describe an unexpected mechanism by which Wnt signals can be downregulated, showing how an extracellular enzyme called Notum renders Wnt inactive.

Detailed biochemical, structural and genetic experiments[1] reveal that Wnt signalling mechanisms are built from unusual elements. When Wnt proteins are made, an acyl group from palmitoleic acid (a monounsaturated form of the lipid palmitic acid) is attached at an evolutionarily conserved serine amino-acid residue, through a carboxyl ester link[3,4]. This modification is made by Porcupine, an enzyme located in a cellular substructure called the endoplasmic reticulum[5]. Such palmitoleoylation is essential for Wnt activity, because it is the acyl group that binds to Frizzled[6] — the transmembrane receptor protein for Wnt — through a hydrophobic cavity in the receptor on target cells. Wnt–Frizzled binding is imperative for receptor activation, and triggers many events in the cell, from modulating gene expression to changing cell shape.

As with many components of the Wnt signalling pathway, Notum was originally discovered in fruit flies, in screens for genes that interact with the Wnt protein Wingless[7,8]. Loss of Notum in flies leads to abnormal wing growth, indicating that Wnt signalling (which drives wing growth and patterning) becomes unrestrained in its absence. Wnt signals also turn on the expression of the gene that encodes Notum, leading to negative feedback regulation that intrinsically limits signalling, as is often the case for such pathways. Initial studies[7,9] suggested that Notum might act as a phospholipase enzyme, cleaving the link between membrane-bound glycoproteins called GPI anchors and glypicans — large polysaccharides that form complexes with extracellular molecules such as Wnt. Cleavage releases glypicans into the extracellular space, decreasing their ability to restrain Wnt activity.

Kakugawa *et al.*[2] unveil a different, previously undocumented function of Notum. The authors start with a structural analysis of human and fly Notum, and find that the protein has the overall structure of a hydrolase enzyme. But it also has a large hydrophobic cavity of around 380 cubic ångströms, which in theory could provide sufficient space for binding by acyl groups with chains of up to 16 carbons — the length of palmitoleic acid. Furthermore, the researchers' analysis of binding between Notum and acyl groups of various lengths and degrees of saturation reveals that, of the longer-chain molecules, only monounsaturated molecules can bind. In other words, the acyl group found on Wnt can form a complex with Notum, whereas lipids with different configurations cannot. In parallel with their binding assays, Kakugawa and colleagues show that Notum enzymatically removes the acyl group from Wnt, thereby rendering the protein inactive. Such an extracellular deacylase activity has never been previously reported.

Hedgehog is another signalling molecule whose activity is modified by lipids. But the authors demonstrate that, unlike Wnt, Hedgehog is not a substrate for Notum. The specificity of Notum for monounsaturated acyl groups provides an explanation for this discrepancy, because the acyl group attached to Hedgehog contains saturated carbon bonds throughout. Kakugawa and co-workers also provide genetic evidence that, in flies, Notum does not interact with Hedgehog signalling *in vivo*. Finally, they show that Notum contains binding sites for polysaccharides such as glypican sugar chains, inviting speculation that glypicans bring together Notum and Wnt — thus modulating the enzymatic interaction of Notum with Wnt, rather than acting as a substrate for Notum to cleave GPI anchors.

Kakugawa and colleagues' discovery adds greatly to our understanding of Wnt signalling, and of the central role of the Wnt lipid group. The authors' results demonstrate how acquisition or loss of the acyl group from palmitoleic acid can adroitly control the activation or deactivation of Wnt signals. The transmembrane

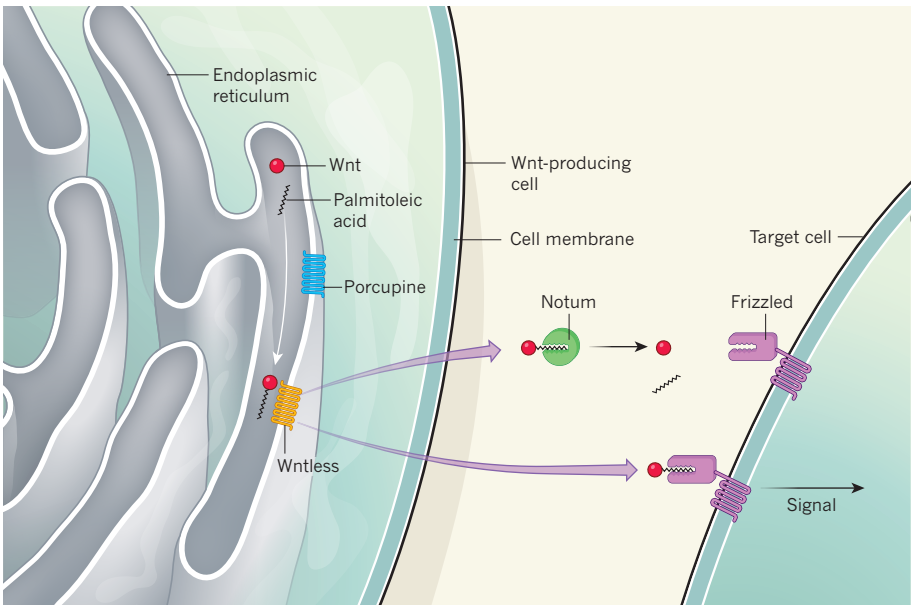

Figure 1 | Notum shoots the messenger in Wnt signalling. In Wnt-producing cells, the Wnt protein is made in a cellular compartment called the endoplasmic reticulum. There, an acyl group from palmitoleic acid is added to Wnt by the membrane-spanning enzyme Porcupine. The Wntless protein then transports palmitoleoylated Wnt out of the cell. Secreted Wnt binds to its receptor protein Frizzled, which spans the membrane of Wnt target cells. This binding depends on the acyl group in Wnt, and triggers an intracellular signalling cascade. Kakugawa *et al.*[2] report that the Wnt–Frizzled interaction is inhibited by the extracellular enzyme Notum, which specifically removes the acyl group from Wnt.

protein Wntless conveys Wnt molecules that have been palmitoleoylated by Porcupine through the cell for secretion[10]. Once secreted, Wnt proteins bind to Frizzled on other cells through the acyl group (Fig. 1).

All of these lipid-related pathway components, including Notum, evolved at around the same time as Wnt. The Wnt protein itself contains a lipid-binding motif called a saposin fold, and it has been speculated[11] that, when Wnt signals initially evolved, they consisted of a lipid–protein complex, with the two becoming covalently linked at a later date. Lipids lie at the heart of Wnt signalling, and can even be viewed as a primordial cell-fate signal because they are also used by organisms such as choanoflagellates, which are located at the base of the animal evolutionary tree[12].

Because enzymes are often good targets for drugs, it might be possible to identify molecules that inhibit the activity of Notum, thereby increasing the strength of Wnt signalling. Wnt proteins can stimulate stem cells to proliferate, so such an approach could have therapeutic value for treating degenerative diseases. Collectively, these findings explain how Notum prevents tissues from growing abnormally or adopting aberrant identities — it shoots the messenger in the Wnt pathway by stripping Wnt proteins of their crucial lipid group. ■

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