A Dedicated Wnt Secretion Factor

Wendy Ching[1] and Roel Nusse[1,*]
[1]Department of Developmental Biology and Howard Hughes Medical Institute, Stanford University School of Medicine, Beckman Center, B271, Stanford, CA 94305, USA
*Contact: rnusse@stanford.edu
DOI 10.1016/j.cell.2006.04.018

The Wnt family of signaling proteins mediates cell-cell communication during development. In this issue of *Cell*, Bänziger et al. (2006) and Bartscherer et al. (2006) identify Wntless/Evi, a multipass transmembrane protein in the secretory pathway of Wnt-producing cells that promotes Wnt secretion.

Patterning complex tissues during the development of an organism is a challenging task. How do cells coordinate their transformation from a sheet of undifferentiated tissue into one that is diverse, differentiated, and functional? As one would expect, this process requires a great deal of intercellular communication. One way that cells communicate with each other is by secreting signaling molecules into the extracellular space. These signals arrive at the surface of target cells, where they attach to their receptors and activate signaling cascades leading to effects such as proliferation, differentiation, or migration. The release, transport, and reception of signaling molecules by cells must be tightly regulated to ensure proper patterning during development. Many studies are directed toward understanding the mechanisms by which this precise control is accomplished.

One important class of signaling proteins is the Wnt family. Wnt proteins are used in various developmental contexts and play key roles in regulating growth, cell-fate specification, and differentiation (reviewed in Logan and Nusse, 2004). They are secreted by cells that express Wnt and have been shown in some cases to act as morphogens, exhibiting both short-range and long-range signaling capabilities (Zecca et al., 1996). Many Wnt pathway components have been identified that are required for reception of the signal in target cells. However, players involved in the crucial role of regulating the production, secretion, and release of the Wnt signal itself are less well understood. Recent studies have begun to shed some light on these processes.

In this issue of *Cell*, two groups describe the finding of a new Wnt pathway component called *wntless* (*wls*) (Bänziger et al., 2006) or *evenness interrupted* (*evi*) (Bartscherer et al., 2006). This gene encodes a new multipass transmembrane protein that is conserved in metazoans from worms to humans. Bänziger and colleagues (2006) identified two alleles of *wls/evi* in a genetic screen for recessive suppressors of a Wnt gain-of-function phenotype in the *Drosophila* eye. Bartscherer and colleagues (2006) identified *wls/evi* in an RNA interference (RNAi) screen for transmembrane proteins that affect Wnt signaling in cultured *Drosophila*

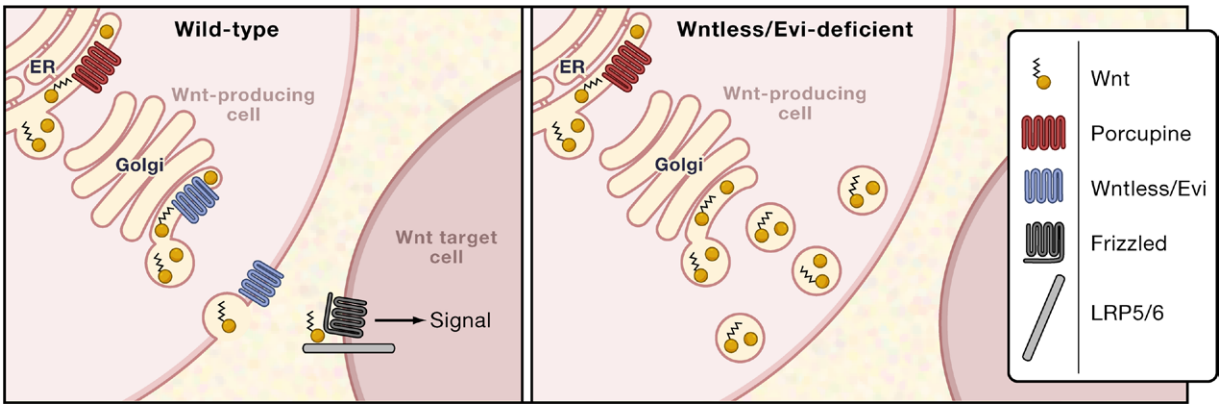

Figure 1. Multipass Transmembrane Proteins in Wnt Production
(Left panel) In Wnt producing cells, the Wnt protein becomes palmitoylated in the endoplasmic reticulum by the acyltransferase enzyme Porcupine. Further transport and secretion of the Wnt protein in secretory vesicles is controlled by the multipass transmembrane protein Wntless/Evi, which is present in the Golgi and/or the plasma membrane. On target cells, the secreted Wnt interacts with its receptors Frizzled and LRP5/6 to initiate signaling.
(Right panel) In the absence of Wntless/Evi, the Wnt protein is retained by producing cells.

cells. Analysis of *wls/evi* mutants in *Drosophila* confirmed its requirement for Wnt-dependent processes during development, such as patterning of the larval epidermis and the wing margin. Clonal analysis in the *Drosophila* wing imaginal disc showed that *wls/evi* is required only in Wnt-producing cells and that loss of Wls/Evi leads to an accumulation of Wnt protein in these cells (see Figure 1). Experiments in cultured cells also established that *wls/evi* RNAi only affects Wnt signaling when applied to Wnt-producing cells and does not affect cells receiving the Wnt signal. In the nematode *Caenorhabditis elegans*, the homolog of *wls/evi*, *mom-3/mig-14* (Rocheleau et al., 1997), is necessary for Wnt signaling. Wnt signaling and secretion are also affected by the absence of *wls/evi* in a human embryonic kidney cell line, suggesting an evolutionarily conserved requirement for this gene.

A key question that arises is whether Wls/Evi is required specifically for the secretion or processing of Wnt proteins or whether it has a more general function in protein secretion. To address this issue, Bänziger et al. (2006) showed that the specific expression of *wls/evi* in *Drosophila* cells where Wnt1 is produced rescued a large fraction of flies that are entirely mutant for *wls/evi*, indicating a specific role for *wls/evi* in Wnt signaling and not in general secretion. In addition, Bartscherer et al. (2006) demonstrated that Hedgehog protein distribution and target-gene expression are not affected in *wls/evi* mutant clones in the *Drosophila* wing disc. They also showed that JAK/STAT signaling is not affected by *wls/evi* RNAi in cultured *Drosophila* cells. Thus, it seems that a new player required specifically for secretion of Wnt proteins has been brought to light.

Further studies to address the molecular function of Wls/Evi are highly anticipated. Bänziger et al. (2006) suggest that Wls/Evi may act in the Golgi or late secretory vesicles to direct Wnt proteins to the appropriate pathways for secretion from the cell (Figure 1). Along these lines, Bartscherer et al. (2006) make the initial observation that the normal subcellular localization of Wnt protein to the apical side of the cell appears to be disrupted in the absence of Wls/Evi in polarized epithelial cells of the *Drosophila* wing disc. Apical secretion of proteins is thought to involve sorting into specialized intracellular secretory pathways within the *trans*-Golgi network, and the apical localization of Wnt proteins in the *Drosophila* embryo has been shown to be important for proper signaling. Intracellular trafficking and differential protein sorting along the secretory pathway are known to regulate the delivery of certain proteins to the plasma membrane for secretion in response to extracellular cues in yeast and in mammalian cells. It is interesting to consider whether intracellular protein sorting may also play a role in regulating the release of developmental signaling molecules.

Another possibility that cannot be ruled out, however, is that Wls/Evi modifies Wnt proteins in a manner similar to the multipass transmembrane endoplasmic reticulum protein Porcupine (Figure 1). Porcupine is one of the only other components in the Wnt signaling pathway known to be required in the Wnt-secreting cell (Kadowaki et al., 1996). Porcupine is homologous to a superfamily of acyltransferase enzymes (Hofmann, 2000) and is thought to play a role in the lipid modification of Wnts (Willert et al., 2003). In the absence of Porcupine, Wnt secretion also appears to be impaired (van den Heuvel et al., 1993), possibly because of misfolding of the unmodified protein. Wnt proteins are known to be glycosylated and acylated, but the presence of other modifications has not been ruled out. If Wls/Evi is an enzyme that modifies Wnt, its absence might lead to abnormal Wnt protein folding and retention in cells as has been observed.

Regardless of its specific mechanism, the discovery of a new Wnt pathway component that plays a critical role in Wnt-producing cells hints at an additional layer of regulation of intercellular signaling. Along with Porcupine, Wls/Evi may be part of a sequence of specific cellular machinery dedicated to controlling the release of Wnt signals. This is reminiscent of the Hedgehog signaling protein, whose release from the cell that produces it is dependent on the multipass transmembrane protein Dispatched (Burke et al., 1999). Dispatched is required specifically for Hedgehog signaling and is believed to contain a sterol-sensing domain that may interact with the cholesterol modification of Hedgehog to facilitate its release from the membrane (Burke et al., 1999). Thus, the secretion of signaling proteins in general may be more complex than previously understood. Dedicated cofactors could have important roles in intracellular protein sorting and posttranslational modification, which may be necessary for the proper production and processing of signaling proteins. Further studies into new pathway components such as these should help us to gain a deeper understanding of how cells are able to tightly regulate the release and subsequent activity of developmental signals in order to orchestrate the remarkable events that lead to the formation of an elaborate multicellular organism.

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